EFFECT OF CHRONIC LEAD EXPOSURE ON THE HEMATOLOGY, BLOOD GLUTATHIONE AND BONE MARROW OF DOGS

by

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS iv

INTRODUCTION 1

EFFECT OF CHRONIC LEAD EXPOSURE ON THE CANINE BONE MARROW 2

Summary 3

Introduction 4

Materials and Methods 4

Results 7

Discussion 9

Figures 14

References 23

EFFECT OF CHRONIC LEAD EXPOSURE ON THE HEMATOLOGY, BLOOD GLUTATHIONE AND BONE MARROW NON-HEME IRON OF DOGS 26

Abstract. 27

Introduction. 28

Materials and Methods 29

Results 32

Discussion. 34

Figures 38

Acknowledgements. 43

References. 44

Page

APPENDICES

A. A Literature Review of Lead Poisoning in Dogs. . 47

B. A Literature Review of Effect of Lead in Bone Marrow 62

C. Individual Animal Data 85

D. Examples of Bone Marrow Cells From Experimental Animals. 105

ABSTRACT

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INTRODUCTION

The undesirable environmental effects of lead have been recognized for many years in industrial workers, people or animals coming into contact with the effluence from factories, and in people or animals exposed to lead-containing products, such as paint. This widespread pollution has resulted in efforts by environmentalists and health orientated professions to minimize lead exposure to humans and populations of animals thought to be at risk.

Sub-clinical lead poisoning is common in humans as well as animals. Because of possible mental retardation in children, subclinical poisoning is gaining world-wide concern.

Lead also affects the central nervous, renal, hepatic, hemopoietic and gastrointestinal systems. It is thought to cause anemia by interferring with enzymes concerned with heme synthesis and by shortening the life span of circulating erythrocytes. Other clinical signs of lead are evidence of abdominal pain, vomiting, constipation, convulsions, emaciation and muscular weakness. Diagnosis is a clinical concern and the establishment of early diagnostic criteria is an important goal of clinical scientists.

The purpose of this study is to evaluate the effect of chronic lead exposure in dogs on the hemopoietic system and to study the possible correlation between blood lead levels and hematologic, biochemical and bone marrow parameters.

EFFECT OF CHRONIC LEAD EXPOSURE ON THE CANINE BONE MARROW

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The authors thank Linda Prebble for her technical assistance in conducting this study.

SUMMARY

The effect of chronic oral lead acetate administration on the canine bone marrow was studied. Two dogs served as control, 4 received 2 mg lead/kg daily and 4 received 5 mg lead/kg daily. Lead dosing was conducted for 13 weeks, after which one-half of each group was treated with calcium ethylene diaminetetracetic acid (CaEDTA). All animals were then observed for another 4 weeks. Blood lead values and bone marrow cytology were monitored weekly during the 18 weeks of study. The lead dosed dogs had a lower rate of weight gain than the control dogs. Clinical signs of toxicosis were observed after 6 weeks in one dog in the high lead group. Anorexia, body weight loss, depression, muscular weakness and trembling were seen. The signs were reversed with CaEDTA treatment. Blood lead levels increased in all lead dosed dogs. Lead caused increases in bone marrow segmented neutrophils and myeloid series cells and increased myeloid:erythroid ratios. Blood lead levels and M:E ratios decreased after cessation of lead administration.

Lead is used in the manufacture of storage batteries, accounting for approximately one-third of the total industrial lead consumption.[1] However, the use of lead as antiknock in fuel additives, the second most prevalent use, is a major source of pollution.[1] The concentration of atmospheric lead has been estimated at 0.0005 ug/M^3 as compared to an average of 0.79 ug/M^3 in urban air.[1] The amount of lead in fresh air has been estimated at 1 to 10 ug/L. Campbell et al[2] has shown that at least three of the enzymes involved in heme biosynthesis are inhibited by lead and may be the cause of lead induced anemia. Goldberg[3] has stated that anemia from lead poisoning is a result of a disorder in heme synthesis.

The purpose of this study was to evaluate the effects of chronic lead exposure on the hemopoietic system of dogs and to document the correlation between blood lead levels and bone marrow cytologic parameters.

Materials and Methods

Ten male beagle dogs, 10 weeks of age, were purchased from Theracon, Inc., Topeka, Kansas. Routine anthelmintic control[a] and vaccination[b] were practised. The dogs were fed dry dog food[c] between 1200 to 2400 hours and offered water ad libitum in individual cages. The dogs were allowed two weeks acclimatization prior to exposure to lead.

They were divided randomly into three groups. Two control dogs (group 1) were given empty gelatin capsules daily. Four dogs (group 2) received 2 mg of lead (as the acetate)/kg[d]

in gelatin capsules daily between 0800-0900 hours. Four dogs (group 3) received 5 mg of lead (as the acetate)/kg orally on the same time schedule. Bone marrow biopsies were taken from each dog before the study was initiated; subsequent biopsies were taken every week from alternately one-half the dogs of every group. Blood was taken from each animal before the study and then every week between 0800-0900 hours. The study lasted for 18 weeks with the lead dosed groups receiving lead the first 13 weeks. After that one-half the dogs from each group were treated with calcium ethylene diaminetetraacetic acid (CaEDTA).[e] Any animal observed sick from the lead exposure was treated immediately with CaEDTA and removed from lead dosing. CaEDTA was given at the rate of 4.5 mg/kg of body weight daily for 5 days. The daily dose was divided into four equal portions and administered subcutaneously after dilution with 5% dextrose solution to a concentration of about 10 mg CaEDTA/ml.

Sample collections.

Blood -- 10-15 ml of blood was taken from the jugular vein of each dog during stabilization and thereafter every week during the 18 weeks of study. Samples were taken between 0800-0900 hours on the same day of bone marrow biopsy. Heparin was used as anticoagulant. The vials were capped immediately and stored in a refrigerator pending blood lead determination. Blood lead was determined by atomic absorption spectrophotometry[f] using standard methods described in the analytical manual for

the Perkin-Elmer 306 instrument; a graphite furnace (HFG) 2200 with deuterium background correction was employed.

Bone marrow -- Animals were lightly anesthetized with intravenous thiamylal sodium[g] at a dose of 0.44 ml of 4% solution/kg body weight. Bone marrow samples were collected alternately from the right and left ileal crests of each dog as described by Coles[4], Perman et al[5], Penny et al[6], and Schalm.[7] Aseptic technique was observed throughout the surgical procedure using a bone marrow biopsy needle[h] 2.5 cm long by 16 g. Smears were made immediately on marked slides. Wrights/Leishman stain was used for staining as outlined by Coles.[8]

Slides were scanned under low power to assess cell type and density and to determine numbers of megakaryocytes; 500 marrow cells[9] were then differentiated under oil immersion. The myeloid:erythroid (M:E) ratios were calculated by enumerating all nucleated cells of the myeloid series as a ratio of all nucleated cells of the erythroid series. The myeloid series included myeloblasts, promyelocytes (progranulocytes), myelocytes, meta, band and segmented forms of neutrophils, eosinophils, and basophils; the erythroid series included rubriblasts, prorubricytes, rubricytes and metarubricytes.

Statistical analysis of data -- The SAS 76 computer[i] statistical package was used for data analysis. Analysis of variance was performed for the 13-week lead dosing period and the 4 weeks post lead dosing. Significance among groups or treatment was tested with Duncan's Multiple Range test at a probability level of 0.05.[10]

Results

Clinical observations. The control dogs (group 1) had a steady increase in body weight throughout the study. These animals remained bright and alert, had very good appetite and were clinically normal.

Group 2 dogs (2 mg lead/kg daily) also had a steady increase in mean body weight, but this was not as consistent or great as in the control group (Fig 1). Although they ate well throughout the study, group 2 dogs were not as bright and alert as the control group.

Group 3 dogs (5 mg lead/kg daily) had consistent mean body weight gains, but at a lower rate than the control animals (Fig 1). One animal in this group showed depression and anorexia after day 42. A tender abdomen, muscular weakness of the hind limbs and loss of weight were also observed. This animal was treated with CaEDTA and improved after one week. Other dogs in this group were not as bright and alert as the control animals.

No seizures were observed in any of the lead dosed groups.

Blood lead level. The control dogs' blood lead levels remained constant during the period of study, but a statistically significant increase in blood lead was observed in both lead dosed groups (Fig 2). The lead dosed groups also had blood levels significantly different from each other ($P < 0.05$), with the highest level in the dogs receiving the high lead dose.

Bone marrow cytology. Control dogs did not have any significant changes of bone marrow cytology. Changes in the bone marrow cytology were observed in the lead dosed groups. Segmented neutrophils increased in both lead dosed groups. Myeloid series cells had a trend to increase in both lead dosed groups (Fig 3), but the degree of increase was not statistically significant. The M:E ratios increased in both lead dosed groups compared to the control group (Fig 4); there was no difference between the two lead dosed groups.

Large binuclear cells of the erythroid series cells (megaloblasts) were frequently seen in the marrow smears of the lead dosed groups. There were many naked nuclei of metarubricytes on marrow smears in these animals. One of the dogs in the low lead dosed group developed a marked hypocellularity of the bone marrow, and for two consecutive biopsies there were very few immature forms of bone marrow cells. No basophilic stippling was observed in the bone marrow smears of either lead dosed group during the study period. Mononuclear bone marrow cells and other cells of the erythroid and myeloid series had no change during the period of study.

Effect of CaEDTA therapy. The clinical signs of toxicosis produced by lead in one animal were reversed after one week of therapy. There were no statistical differences in the weekly blood lead levels of animals treated with CaEDTA and those not so treated. Blood lead levels dropped in both instances with cessation of lead administration (Fig 2). Myeloid series cells

and segmented neutrophils remained statistically elevated in CaEDTA treated animals; however this was not of practical significance due to the small sample size.

Discussion

The loss of weight, anorexia, tender abdomen and muscle weakness observed in one of the high lead dosed dogs was also reported by other authors[1,11-13] in canine lead intoxication. The lesser degree of severity in this study may be due to the low dose levels used in this study, and the fact that lead is poorly absorbed from oral administration.[1] Toxic levels of lead cause anorexia, colic, and weight loss by astringent action on the mucosa of gastrointestinal tract, leading to gastroenteritis; hence subsequent malabsorption of nutrients and gradual emaciation results. Beliles[1] suggests that weakness of extensor muscles, as well as the gastrointestinal signs, may be related to peripheral neuropathy and not to a direct effect of lead on the intestinal mucosa. Other reported gastrointestinal signs (vomiting and constipation[11-13]) were not observed in this study. The absence of convulsions and tremors[11,13,14] may have been due to the low dose or limited exposure. However, convulsions can be periodic and thus difficult to observe.

Treatment of the one ill dog with CaEDTA reversed the clinical signs promptly. The antidote acts by chelating lead circulating in the blood, making it available for renal excretion and thereby decreasing the blood lead level. In our

study, CaEDTA did not appear to reduce blood lead levels after continuous lead exposure was stopped. This was probably due to redistribution of lead to blood from the heavily burdened body tissues, such as bone. The fact that 3 days elapsed between the last CaEDTA treatment and the subsequent blood lead determination allowed for this redistribution and equilibrium.

Blood lead concentrations are expected to increase with dosing level and duration[14,15] as observed in this study. Zook and Carpenter[14] described blood lead values of 60 ug or more of lead/100 ml of blood (0.6 ppm) as virtually diagnostic of lead poisoning in dogs. Blood lead values of 40-50 ug/100 ml (0.4-0.5 ppm) are reported to indicate lead poisoning if associated with typical signs and hematologic findings.[14] Although blood lead levels in our study increased with intensity of lead dosing, only one dog had clinical signs of intoxication. Despite the presence of blood levels considerably above the diagnostic level of 60 ug/100 ml blood in the high lead dosed dogs for several weeks, the other 3 dogs in this group did not exhibit any clinical signs of poisoning other than reduced body weight gain. This suggests that blood lead levels at or in excess of the suggested diagnostic limit are not necessarily associated with the expected overt clinical signs of poisoning.

The changes observed in the bone marrow of the lead dosed dogs were increased numbers of segmented neutrophils and myeloid series cells and elevated M:E ratios. Many

workers[3,16,17] have shown that lead may affect the early precursors of the erythroid series. Morse et al[16] suggests that the effects of lead on erythropoiesis in mice are due to intramedullary death of intermediate normoblasts. Waldron[17] proposes that lead has a profound effect on red cell precursors in the bone marrow, producing nonspecific morphologic changes.

Nearly fifty times as much lead is found in human bone marrow as in peripheral blood; 1.4 mg lead occurs/100 gm fresh marrow, as compared to only 0.15 mg lead/100 gm in liver and 0.10 mg lead/100 gm in kidney. A patient whose blood lead varies between 70 and 130 ug/100 ml blood will have a bone marrow concentration of 4.2 to 9.2 mg lead/100 gm[17,18]. It is therefore logical that the effects of lead should be demonstrable in the bone marrow, where young cells are most susceptible to injury.

Increased M:E ratios were observed after day 63 in both lead dosed groups; elevated M:E ratios have not previously been reported in dogs exposed to lead. Lead may have a direct suppressing effect on the erythroid series, allowing the myeloid series cells to dominate, or it may interfere with maturation and release of segmented neutrophils from the bone marrow into the circulation. A study of myeloid and segmented neutrophil kinetics could investigate this concept further. Calhoun[19] found that four anemic horses with lead poisoning had M:E ratios falling below 0.5. The values reflected active erythrogenosis or decreased myeloid production. He found that the number of rubricytes and metarubricytes were increased.

The effect of lead on M:E ratios resembles the effect of chloramphenicol on the hemopoietic system of the dog and cat. Penny et al[20,21] showed that chloramphenicol increased the M:E ratios significantly in dogs and cats and produced severe depression of the bone marrow. Watson[22] also observed an increased M:E ratio in a dog with chloramphenicol intoxication. The increased M:E ratios in the lead dosed groups in this study were not as high as those observed with chloramphenicol. This suggests that lead does not exert the same quantitative effects on bone marrow erythroid cells as chloramphenicol does.

The M:E ratios in our study declined gradually after cessation of lead administration, indicating that this effect was lead related. However, a slightly increased M:E ratio should not be considered diagnostic of lead poisoning unless other parameters, such as blood lead levels, are also elevated.

The marked hypocellularity observed in one animal in the lead dosed group is difficult to explain. Most workers[16,17] have found general erythroid hypoplasia with lead toxicity. Individual animal variation must not be overlooked in this instance, since only one animal was observed to have poor capacity for bone marrow regeneration.

There was no basophilic stippling observed in the bone marrow smears of the lead dosed dogs. Waldron[17] suggests that a diagnosis of lead intoxication is not dependent upon the findings of basophilic stippling in bone marrow or

peripheral blocd smears. Thus, although basophilic stippling is reported to be consistent with lead poisoning, its absence should not rule out that possibility.

Binuclear forms of the erythroid series were observed in our study. This is similar to early findings cited by Waldron[17], who described these cells as megaloblasts. Binuclear forms may result from abnormalities in mitosis and could be caused by lead.[17] There were many naked nuclei from the bone marrow smears of the lead dosed dogs. These nuclei are extruded from metarubricytes, and their increased number may have been due to the effect of lead on maturation of these cells.[7]

Fig 1 — Body weights (kg) during the 13-week dosing period in groups of dogs receiving 0, 2 or 5 mg lead/kg body weight daily.

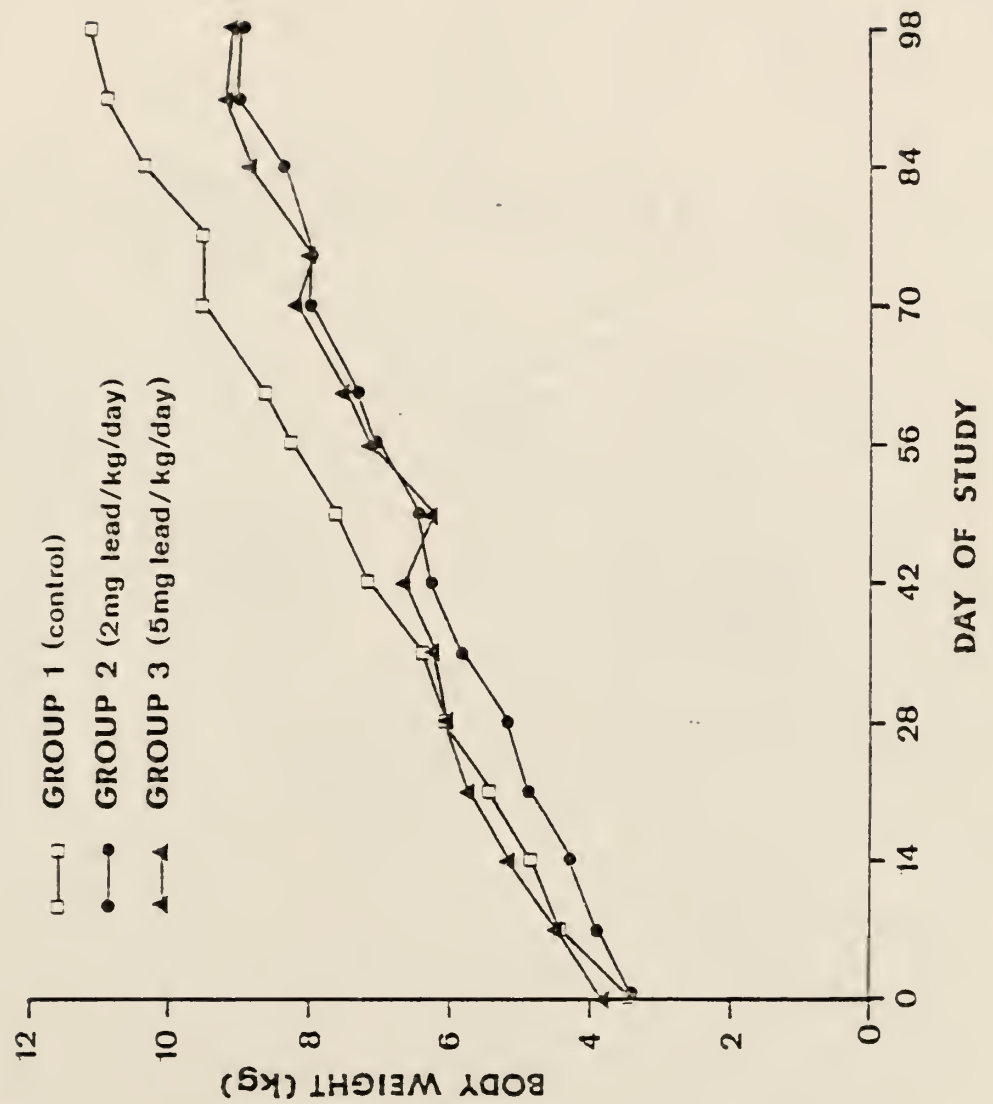

GROUP 1 (control)
GROUP 2 (2mg lead/kg/day)
GROUP 3 (5mg lead/kg/day)
BODY WEIGHT (kg)
DAY OF STUDY
0
2
4
6
8
10
12
0
14
28
42
56
70
84
98

Fig 2 — Blood lead levels (ug/100 ml) during 13-week dosing and 4-week post dosing periods from groups of dogs receiving 0 (group 1), 2 (group 2) or 5 (group 3) mg lead/kg body weight daily.

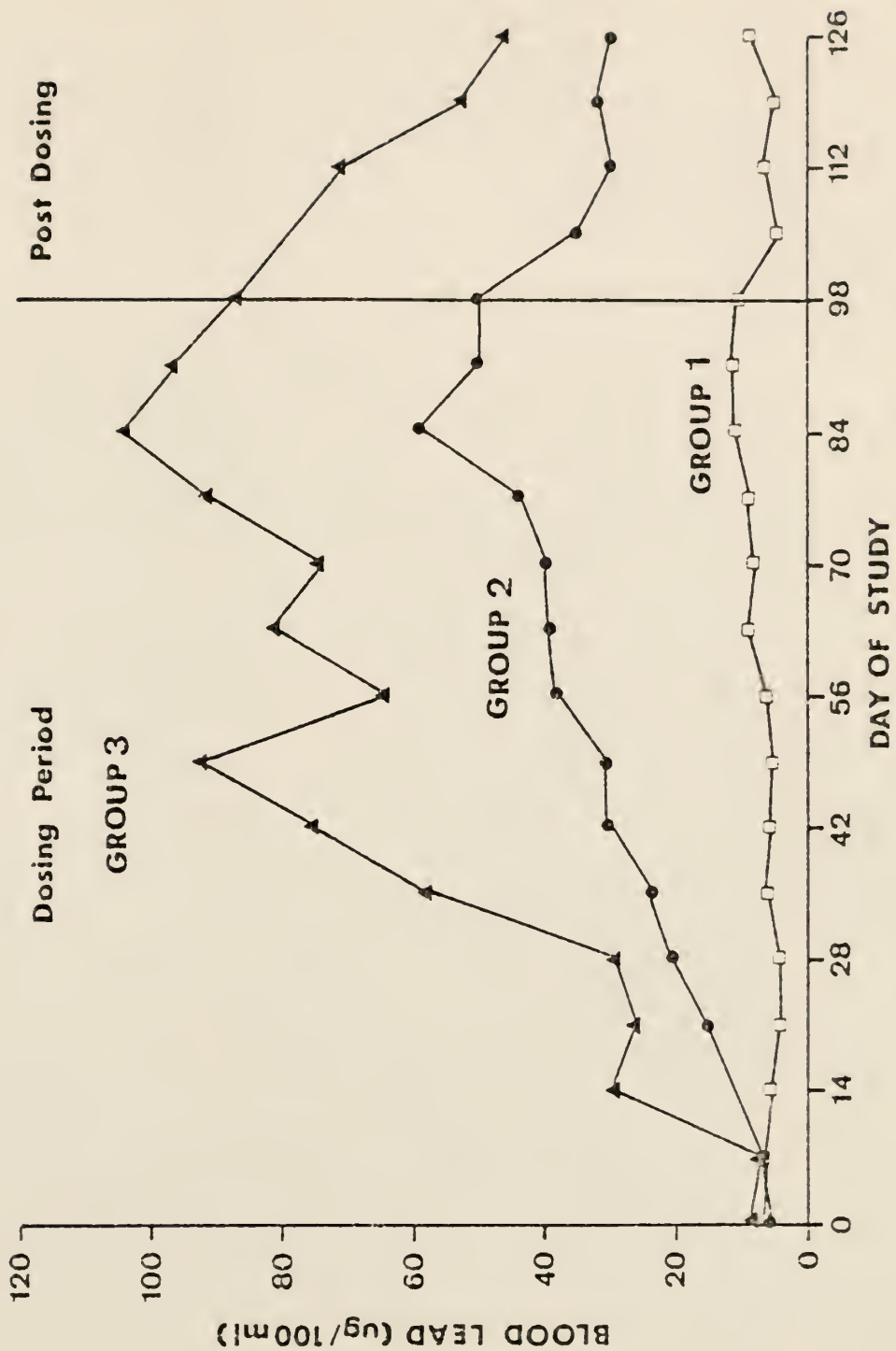

Dosing Period
Post Dosing
GROUP 3
GROUP 2
GROUP 1
BLOOD LEAD (ug/100ml)
DAY OF STUDY
0
20
40
60
80
100
120
0
14
28
42
56
70
84
98
112
126

Fig 3 — Myeloid series cells/500 bone marrow cells counted during 13-week dosing and 4-week post dosing from groups of dogs receiving 0 (group 1), 2 (group 2) or 5 (group 3) mg lead/kg body weight daily.

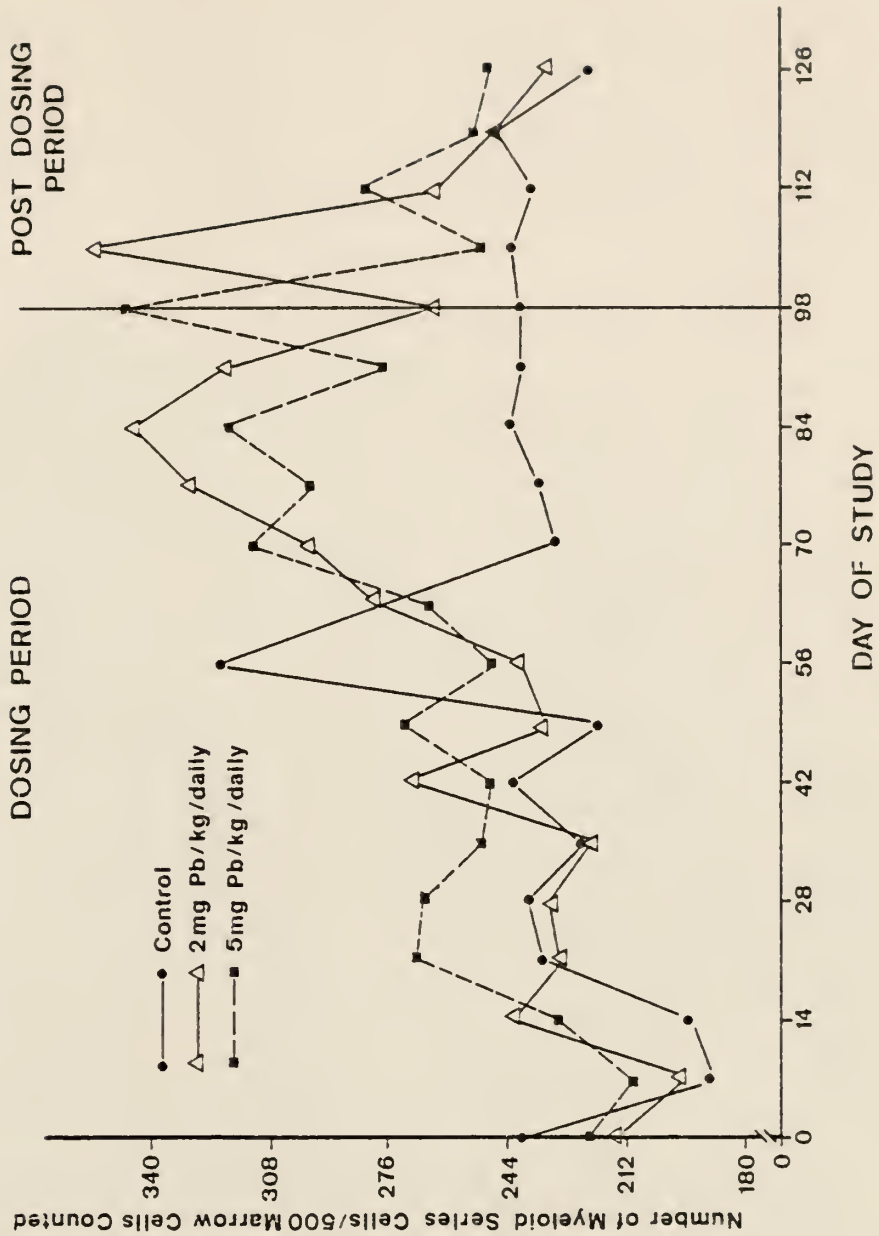
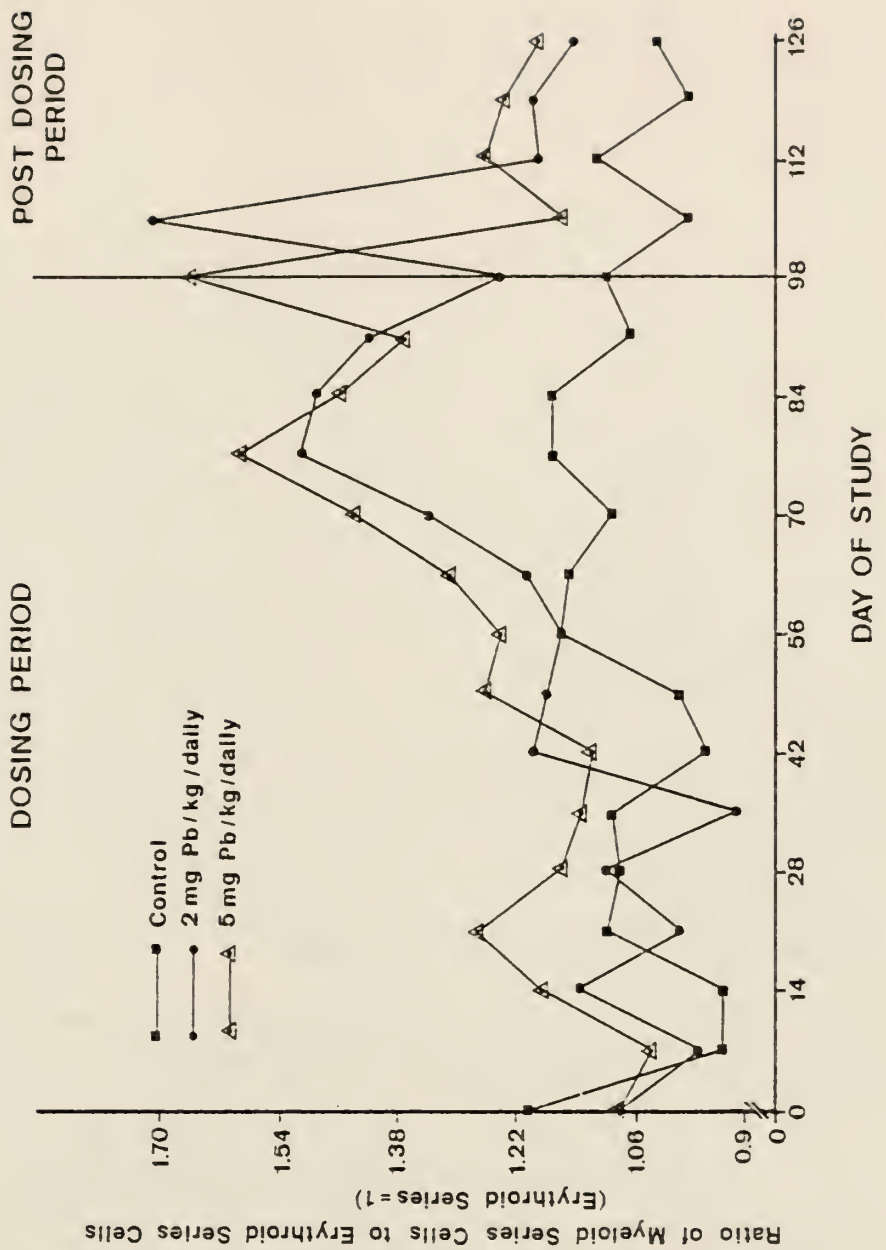

POST DOSING PERIOD
DOSING PERIOD
Control
2mg Pb/kg/daily
5mg Pb/kg/daily
Number of Myeloid Series Cells/500 Marrow Cells Counted
340
308
276
244
212
180
0
0
14
28
42
56
70
84
98
112
126
DAY OF STUDY

Fig 4 — Ratio of myeloid series cells to erythroid series cells during 13-week dosing and 4-week post dosing periods from groups of dogs receiving 0 (group 1), 2 (group 2) and 5 (group 3) mg lead/kg body weight daily.


POST DOSING PERIOD
DOSING PERIOD
Control
2 mg Pb/kg/daily
5 mg Pb/kg/daily
Ratio of Myeloid Series Cells to Erythroid Series Cells
(Erythroid Series = 1)
1.70
1.54
1.38
1.22
1.06
0.9
0
0
14
28
42
56
70
84
98
112
126
DAY OF STUDY

[a]Task dewormer, Shell Chemicals, San Ramon, CA.

[b]Enduracell dhp (Norden DM), Norden, Lincoln, NE.

[c]Purina Dog Food, Ralston Purina Comp, St. Louis, MO.

[d]Lead acetate, Mallinckrodt, Inc., St. Louis, MO.

[e]Havidole, Haver-Lockhart Laboratories, Shawnee, KS.

[f]Atomic Absorption Spectrophotometer (Ass), Perkin-Elmer, Norwalk, Conn.

[g]Surital, Parke-Davis, Detroit, Mich.

[h]Biopsy needle, Lue-Lock Hub, McGraw Park, Ill.

[i]SAS 76 Computer, SAS Inst. Inc., Raleigh, N.C.

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EFFECT OF CHRONIC LEAD EXPOSURE ON THE HEMATOLOGY, BLOOD GLUTATHIONE AND BONE MARROW NON-HEME IRON OF DOGS

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EFFECT OF CHRONIC LEAD EXPOSURE ON THE HEMATOLOGY, BLOOD GLUTATHIONE AND BONE MARROW NON-HEME IRON OF DOGS

by

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Abstract: Ten clinically normal male beagle dogs were used in the study. Two dogs served as control, 4 received 2 mg lead/kg/daily and 4 received 5 mg lead/kg/daily. Lead was administered for 13 weeks, after which one-half of each experimental group was treated with calcium ethylene diaminetetraacetate (CaEDTA) for 5 days. All animals were then monitored for another 4 weeks. Blood lead levels, hematology, blood glutathione concentration, and the number of bone marrow cells with stainable iron granules were measured weekly during the 18-week experimental period. Clinical signs of poisoning were observed only in one dog in the high dose group after 6 weeks. The signs included emaciation, anorexia, muscular weakness, evidence of abdominal pain and depression. These signs were reversed with cessation of lead dosing and CaEDTA treatment. Blood lead levels and the number of marrow cells with non-heme iron increased in both lead-dosed groups; nucleated red blood cells increased only in high lead dosed group. There was a trend for an increased packed cell volume in all groups; however, the high lead dosed group did not increase as fast. No significant changes were observed in blood

glutathione concentration and in other hematologic parameters. There were no differences in the parameters studied between the dogs treated with CaEDTA and those not so treated. Blood lead levels and the number of nucleated red blood cells decreased after cessation of lead administration and the number of marrow cells with iron also tended to decrease after lead removal.

Key-words: Lead exposure - blood lead levels - hematology - blood glutathione - iron granules - dogs.

Lead poisoning is a common syndrome in domestic dogs, but is infrequently recognized due to the difficulty in detecting early clinical signs (Zook and Carpenter 1974). Zook (1974, 1972b) reported blood lead levels and hematologic findings. He suggested that blood lead levels of 60 ug or more of lead/100 ml whole blood was diagnostic of lead intoxication if the accompanying clinical signs and hematological parameters are present. Hematological parameters reported included elevated white blood cell count (WBC) due to a left shift, neutrophilic leukocytosis, normal hematocrit, eosinopenia, nucleated RBC in peripheral smears and basophilic stippling. The clinical signs of tender abdomen, depression, emaciation, anorexia and muscular weakness were also reported by Beliles (1972), Buck et al. (1976), Stowe et al. (1973), and Zook and Carpenter (1969) in cases of lead poisoning. No relationship between blood glutathione concentration and

blood lead levels has been reported. Brady et al. (1978) reported decreased glutathione concentration in fasted beagle dogs. Deposition of stainable non-heme iron granules in the bone marrow has been reported by Kaplan et al. (1954), Kerr (1957), Beutler et al. (1958), Waldron (1966), Albahary (1972) and Ritcher (1978) in cases of lead poisoning.

The present experiments were conducted to study the effects of chronic lead exposure on the hematology, blood glutathione concentration, and iron granules in bone marrow of dogs and to evaluate the use of these parameters in the early diagnosis of lead intoxication.

MATERIALS AND METHODS

Experimental Animals and Protocol

Ten male beagle dogs, 10 weeks of age, were purchased from Theracon, Inc., Topeka, Kansas. Routine anthelmintic administration and vaccination was practised. The dogs were offered dry Purina dog food daily between 1200-2400 hours and given water ad libitum in individual cages. The dogs were allowed two weeks acclimatization prior to exposure to lead in the form of acetate. Lead acetate was manufactured by Mallinckrodt, Inc., St. Louis, Mo., U.S.A.

Dogs were divided randomly into three groups. Two control dogs (group A) were given empty gelatin capsules daily. Four animals (group B) received 2 mg lead/kg in gelatin capsules daily between 0800-0900 hours. Four dogs (group C) received 5 mg lead/kg orally on the same time schedule.

The study lasted 18 weeks with lead-dosed groups receiving lead for the first 13 weeks. Any animal which became clinically sick from lead exposure during the first 13 weeks was removed from lead dosing and treated with CaEDTA (HAVIDOTE, Haver-Lockhart Laboratories, Shawnee Mission, Kansas, U.S.A.) for 5 days. After the 13 weeks of lead dosing, half of the animals from each group were treated with CaEDTA. All animals were then monitored for 4 weeks. Bone marrow samples were collected on alternate weeks from one-half the dogs in every group while blood was collected from each animal weekly.

Sample Collections and Analysis

Blood. Between 10-15 ml of blood was taken from the jugular vein of each dog during the stabilization period and thereafter every week for 18 weeks between 0800-0900 hours. Sodium ethylene diamine tetraacetate (EDTA) (manufactured by Sherwood Medical Industries, St. Louis, Mo., U.S.A.) was used as anticoagulant for hematology samples and heparin (manufactured by Richer Laboratories, Inc., Northridge, California, U.S.A.) was used similarly for blood collected for lead and glutathione determinations. The vials were capped immediately pending hematologic evaluation and assays for lead and glutathione concentration.

The hematologic parameters--hematocrit (PCV), total white blood cell count (WBC), total red blood cell count (RBC), hemoglobin concentration (Hb) and differential counts--were determined by standard methods (Schalm et al. 1975).

Blood lead values were determined as ug/100 ml of whole blood by atomic absorption spectrophotometry (ASS) using methods described in the analytical manual for the Perkin-Elmer 306 instrument, and using a graphite furnace 2200 with deuterium background correction (manufactured by Perkin-Elmer, Norwalk, Conn., U.S.A.).

Blood glutathione was determined by the method of Beutler et al. (1963) using a double-beam spectrophotometer (manufactured by Hitachi-Perkin-Elmer, Norwalk, Conn., U.S.A.).

Bone marrow collection and iron staining. Animals were anesthetized with thiamylal sodium (SURITAL, manufactured by Parke-Davis, Detroit, Mich., U.S.A.) at a dose of 1 ml of 4% solution per 2.3 kg body weight. Bone marrow samples were collected alternately from the right and left ileal crests of each dog as described by Coles (1967), Perman et al. (1974), Penny et al. (1970) and Schalm (1975). Aseptic technique was observed employing a 16 g 2.54 cm long bone marrow biopsy needle (supplied by Lue-Lock Hub, McGraw Park, Ill., U.S.A.).

Marrow smears were made immediately on marked slides and were stained for Prussian blue reaction (PB) by application of potassium ferrocyanide and hydrochloric acid as described in the method of Beutler (1972). Stained smears were examined under oil immersion. A portion of the smear in which marrow cells were definitely spaced was uniformly and systematically examined for identification of iron containing cells (sideroblasts). Each iron-containing cell was tallied and the mean of each group was calculated and compared.

CaEDTA Treatment

CaEDTA was given at the rate of 111 mg/kg of body weight for 5 days. The daily dose was divided into four equal parts and administered subcutaneously after dilution with 5% dextrose to a concentration of 10 mg CaEDTA/ml. All animals were then monitored for 4 weeks.

Statistical Analysis

The SAS 76 computer statistical package (SAS Institute, Inc., P.O. Box 10066, Raleigh, N.C. 27605, U.S.A.) was used for data analysis. Analysis of variance was performed for blood lead and glutathione concentration and for the hematologic parameters during the 13-week lead dosing and 4-week post-lead dosing periods. The significance among the groups or effect of EDTA treatment was tested with Duncan's Multiple Range Test as described by Snedecor and Cochran (1971) at a probability level of 0.05. Mean weekly numbers of marrow cells with iron granules from each group was tested by linear regression (Snedecor and Cochran 1971) at a probability level of 0.05.

RESULTS

Blood lead levels. The control dogs' blood lead levels remained constant during the whole period of study. A statistically significant increase ($P < 0.01$) in blood lead was observed in both lead-dosed groups (Fig 1) compared to control animals. The lead-dosed groups had blood lead levels significantly different from one another.

Clinical observations. Control animals had a steady increase in body weight throughout the study. These animals remained bright and alert, and had good appetite. The lead-dosed dogs gained steadily in mean body weight, but less than control dogs.

One dog in the high lead dose (5 mg Pb/kg) became clinically ill on day 42 and was treated with CaEDTA. Clinical signs observed were abdominal pain, depression, emaciation and muscular weakness of the hind limbs. Other animals in this group did not show any of these signs of toxicosis.

Convulsions were not observed in any of the lead-dosed dogs.

Hematologic findings. The hematologic parameters (WBC, RBC, Hb, and differential counts) were not different between the groups. The PCV increased in all groups, but did not increase as rapidly in high lead dosed group (Fig 2). This difference was not, however, statistically significant. There was an increased number of nucleated red blood cells (NRBC) in the high lead dosed group (Fig 3). No clinical anemia or basophilic stippling was observed in both lead-dosed groups.

Blood glutathione levels. No significant differences were observed in blood glutathione (mg/gm Hb) between groups ($P > 0.05$); however, there were considerable weekly mean variations within the groups (Fig 4).

Iron granules. There was a trend of an increasing number of bone marrow cells with iron granules in both lead-dosed groups after day 56 (Fig 5).

Effect of CaEDTA. The clinical signs of toxicosis produced by 5 mg lead/kg in one animal were reversed one week after CaEDTA therapy.

There were no significant differences in the parameters studied of the dogs treated with CaEDTA and those not so treated. Blood lead levels decreased with cessation of lead administration in both cases. Hematology, blood glutathione concentration, and the numbers of bone marrow cells with iron granules did not show any effect due to CaEDTA.

DISCUSSION

The blood lead concentration increased with level and duration of dosing. Although blood levels increased, there were no clinical signs in all, but one of the lead-dosed animals. Lead causes anorexia, evidence of abdominal pain, and weight loss by its astringent action on the gastrointestinal mucosa, leading to poor absorption of nutrients and gradual loss of weight. The vomiting and constipation reported by Buck et al. (1976), Stowe et al. (1973) and Zook (1974) were not observed. The absence of the seizures (Buck et al. 1976) is difficult to explain, but since the seizures are reported to be periodic, they may have been sporadic and not observed. A higher body lead burden may be required to produce nervous activity.

No clinical anemia was observed in this study, but anemia has been reported (Stowe et al. 1973). Anemia has been reported to be mild and hypochromic, with hemoglobin concentration rarely falling below 9 gm% (Waldrom 1966). There were no

basophilic stippled cells observed; although these have been reported, they are by no means a constant accompaniment of lead poisoning (Waldrom 1966). The reported increased number of NRBC was observed in our high lead-dosed group. Stowe et al. (1973) reported a significant anemia in dogs fed a low calcium-phosphate diet with dietary lead dosing. He also reported a decreased hemoglobin concentration, decreased PCV, and increased numbers of NRBC. The absence of anemia observed here may have been due to the normal levels of calcium and phosphate in the diet of the dogs used. In this study we observed a trend of increasing PCV in all groups, but this was not as rapid in the high lead dosed group.

The mechanism by which lead causes anemia is not well understood. Lead is thought to increase fragility of erythrocytes (Waldron 1966). Berk et al. (1970) reported a shortened mean red cell survival time and concluded that the hemolytic effect of lead was a direct action on mature red blood cells. The increased mechanical fragility and shortened life span may be the cause of the hemolytic anemia.

Nearly 50 times as much lead is found in bone marrow as peripheral blood (Albahany 1972); it is therefore logical that the effects of lead should be demonstrable in the marrow and on heme synthesis in that tissue. At least two enzymes in heme synthesis may be affected by lead (Campbell et al. 1977). Delta-aminolevulinic acid dehydratase (ALA-D) is one of the most lead-sensitive enzymes. Ferrochelatase (heme

synthetase) (Ortzonsek 1967) which catalyzes the incorporation of iron into the porphyrin structure is also inhibited by lead. Lead inhibits these enzymes by combining with their sulfhydryl (-SH) groups (Waldron 1966; Albahany 1972). Defective hemoglobin synthesis due to one or both of these inhibitions may therefore cause the reported anemia.

Glutathione was not significantly changed during the 18-week study; however, considerable mean weekly variations occurred in all groups (Fig 3). Although Brady et al. (1978) showed a significant glutathione decrease occurred in fasted beagle dogs, no previous work has been reported on variations in blood levels of glutathione in response to lead. Glutathione is a tripeptide of three amino acids (glycine, glutamic acid, and cystine) connected with sulfhydryl bridges. Almost all the reduced glutathione is found within the erythrocytes; its activity is decreased in individuals who are sensitive to the hemolytic action of drugs like acetylphenylhydrazine (Beutler et al. 1955; Beutler 1957). Glutathione is thought necessary to maintain stability of erythrocyte membranes, hence it is possible that chemicals like lead may combine with its sulfhydryl groups and predispose the red blood cells to hemolysis. The effects of lead on glutathione may be the cause of erythrocyte hemolysis reported with lead toxicity (Berk et al. 1970).

Iron accumulates in bone marrow cells due to the effects of lead on the heme synthetase enzyme. PB-reaction iron granules have been reported by Ritcher (1978) in human patients

with lead poisoning. Other cells (leukocytes and macrophages) can also accumulate iron granules, principally as ferritin and hemosiderin. Iron granules are frequently seen in normoblasts (sideroblasts) and macrophages in hemolytic anemias (Tschudi et al. 1977). The number of bone marrow cells with the PB-reaction showed a decreasing trend following therapy with CaEDTA. Ritcher (1978) reported that ferritin or hemosiderin vanished within 4 days from human bone marrow when lead administration was terminated. This did not occur in our dogs.

EFFECT OF CHRONIC LEAD EXPOSURE ON THE HEMATOLOGY, BLOOD GLUTATHIONE AND BONE MARROW NON-HEME IRON OF DOGS

By Eric S. Mitema, F. W. Oehme, L. Penumarthy

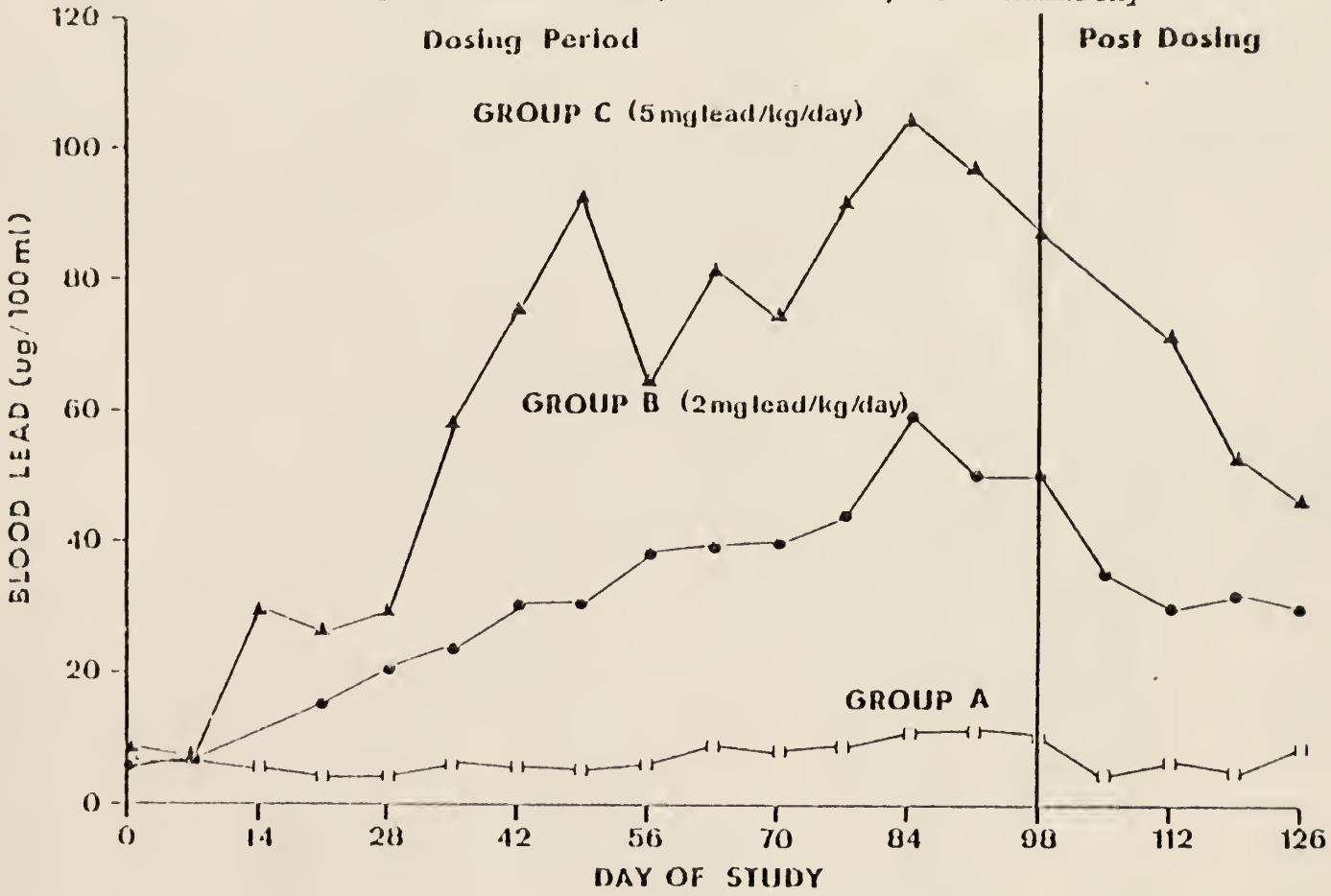

Fig. 1. Blood lead levels (ug/100 ml) during 13-week dosing and 4-week post-dosing periods for groups of dogs receiving 0, 2 or 5 mg lead/kg body weight daily.

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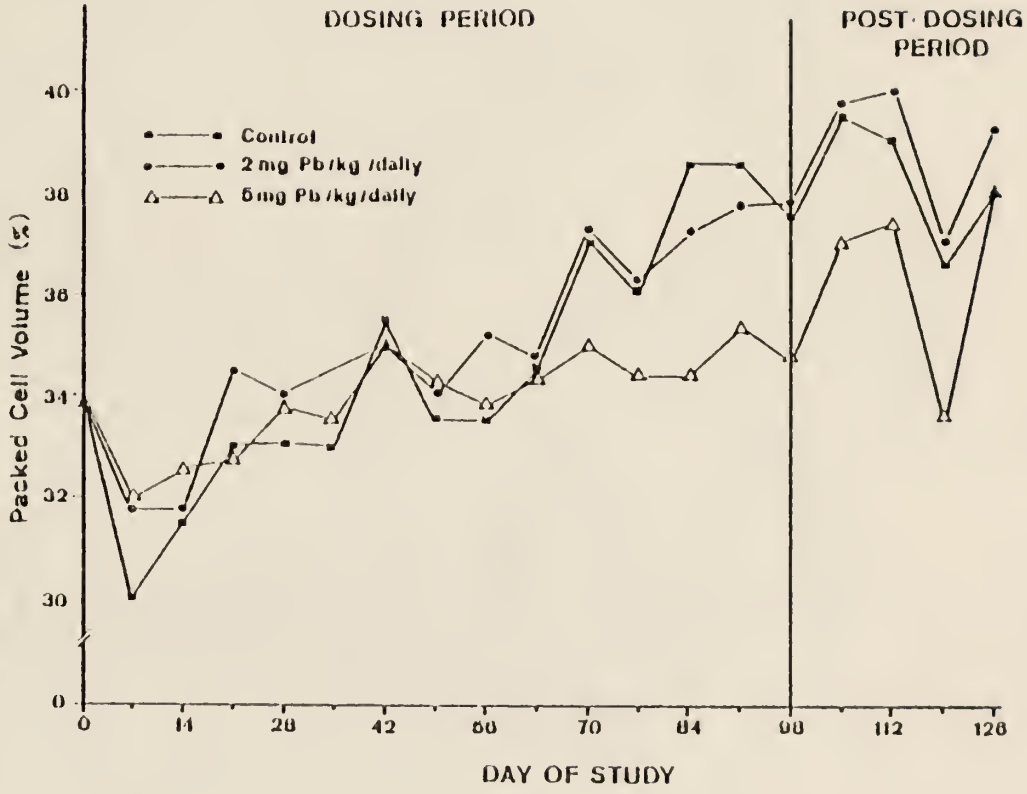

Fig. 2. Packed cell volume (%) during 13-week dosing and 4-week post-dosing period for groups of dogs receiving 0 (group A), 2 (group B) or 5 (group C) mg lead/kg of body weight.

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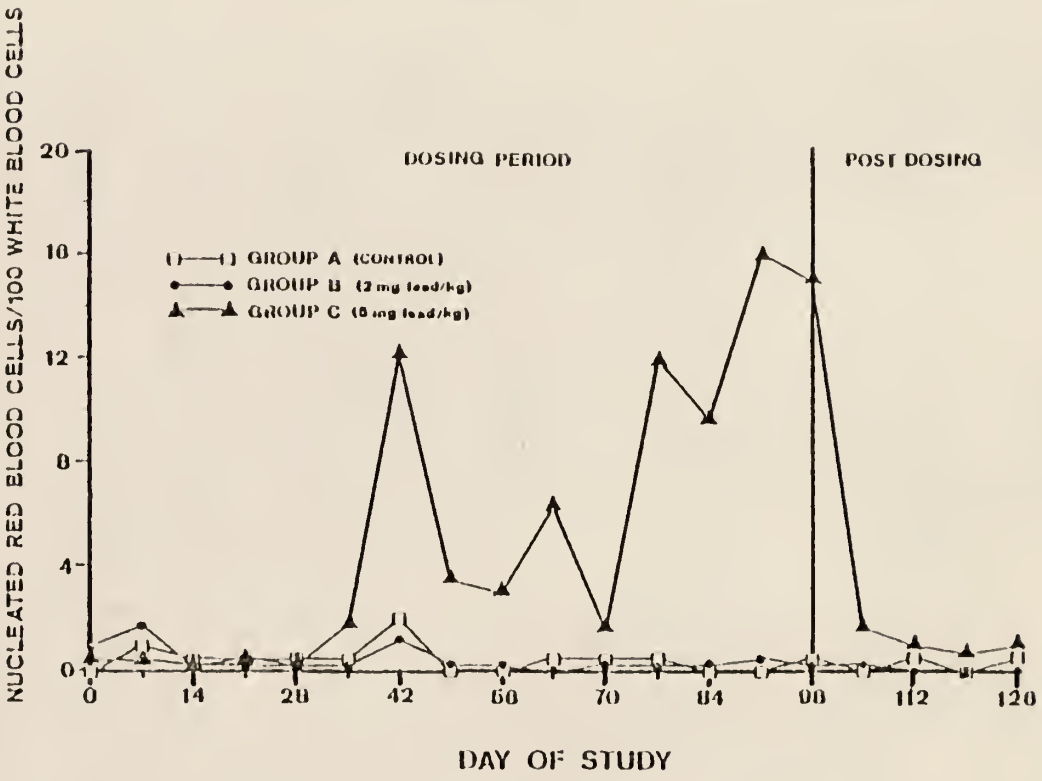

Fig. 3. Nucleated red blood cells/100 white blood cells during 13-week dosing and 4-week post dosing periods for groups of dogs receiving 0, 2 or 5 mg lead/kg body weight daily.

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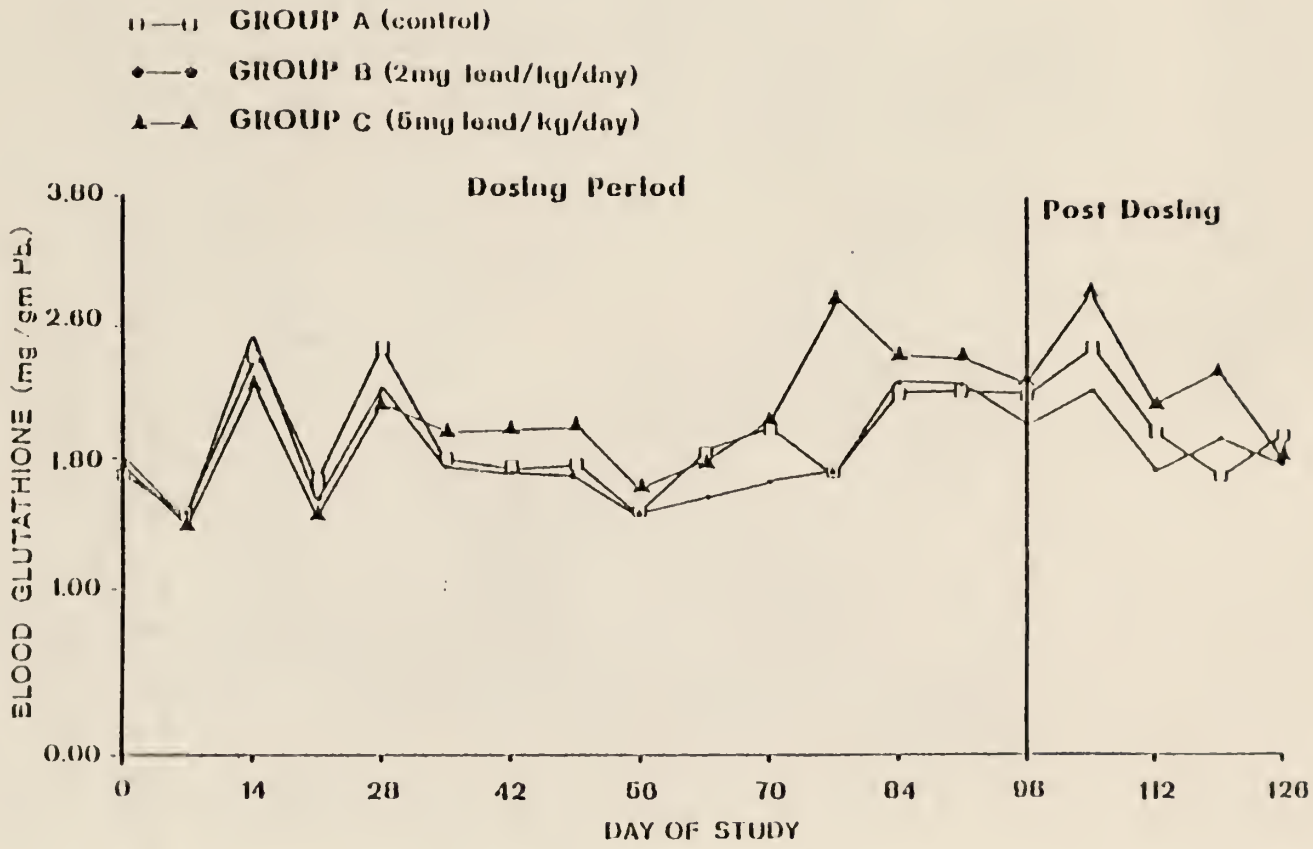

Fig. 4. Blood glutathione concentration (mg/gm Hb) during 13-week dosing and 4-week post dosing periods for dogs receiving 0, 2 or 5 mg lead/kg body weight daily.

EFFECT OF CHRONIC LEAD EXPOSURE ON THE HEMATOLOGY, BLOOD GLUTATHIONE AND BONE MARROW NON-HEME IRON OF DOGS

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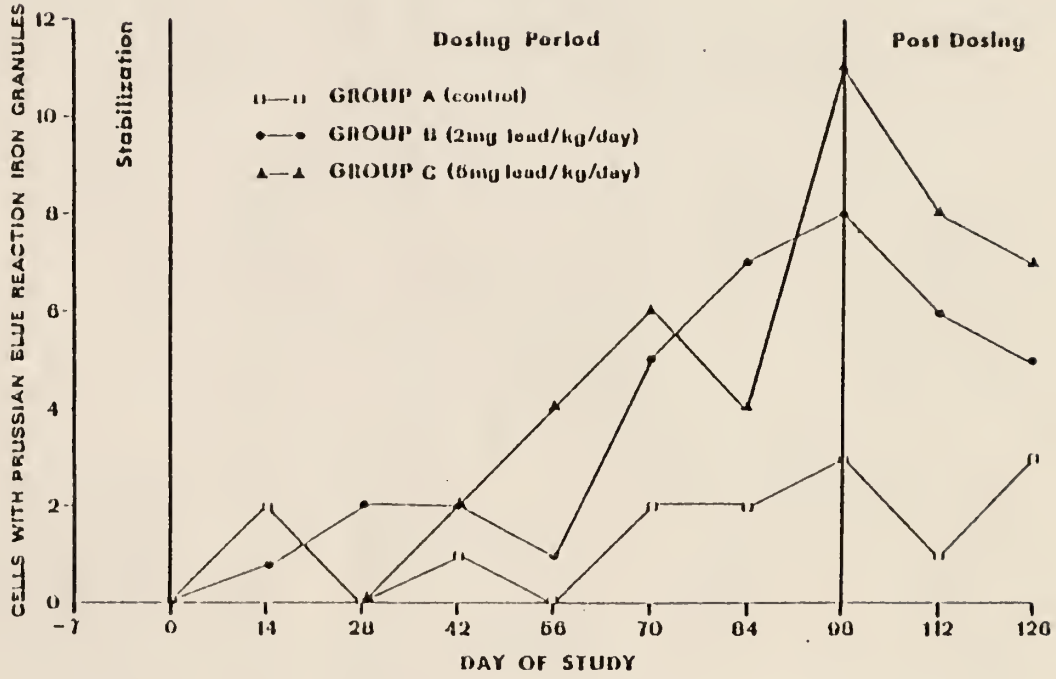

Fig. 5. Number of bone marrow cells with Prussian blue reaction iron granules during 1-week stabilization, 13-week dosing and 4-week post dosing periods for groups of dogs receiving 0, 2 or 5 mg lead/kg body weight daily.

Acknowledgments

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A LITERATURE REVIEW OF LEAD POISONING IN DOGS

INCIDENCE

All species are susceptible to lead poisoning, but it is most frequent in cattle, horses, waterfowl, dogs and children. In dogs, it is most frequent in those less than one year of age, and this may be due to their bizzare chewing and eating habits.[1-3,10] It has been reported that 4% of hospitalized dogs under 6 months of age at Angell Memorial Animal Hospital in Boston had lead poisoning.[2,10,12]

Lead poisoning is seen mainly in summer and fall. This may be due to a higher level of vitamin D which increases intestinal absorption of lead or because dogs spend more time outside during these seasons, where lead sources are more readily available.[1,2,3,4,10] Dogs in slums have higher incidence of subclinical poisoning.[5] These dogs may become clinically sick when they are stressed. Lead deposits in the body, particularly large doses, can be mobilized during the course of canine distemper infection.[6,10]

SOURCES

The most common source of lead poisoning is lead paint.[1-5,10,11,13] Interiors of homes covered before 1940 often contain layers of lead-based paint. Exteriors of buildings and fences are frequently covered with lead paints. Lead chromate is one of the common constituents of paints.

Soil and vegetation may become contaminated with lead

from painted structures, industrial contamination or automobile exhaust.[4] The concentration of lead in the soils, exclusive of areas near lead deposits, has a range of 2 to 200 ppm, with an approximate mean of 16 ppm.[13] Plants can translocate soluble lead through their roots to leaves and fruits.

The natural concentration of atmospheric lead has been estimated at 0.0005 ug/M^3 as compared to the average of 0.79 ug/M^3 in urban air. The amount in fresh air has been estimated at about 1 to 10 ug/L^{13}. Some pet foods may contain up to 7 ppm of lead.[2] Lead pipes can be another readily available source of lead in water, if the water is acidic.

Other sources of lead include linoleum, batteries, plumbing material, putty, lead foil, solder, golf balls, certain roofing materials, lubricants, rug pads, improperly glazed ceramic water bowls, lead weights, fishing sinkers, drapery weights and toys.[1,2] Newspapers and magazines used as litter may result in doses of lead when ingested. The lead content varies according to the ink, paper and printing process. In one study, newspapers averaged 8 ppm per page; black and white printed pages of magazines 10 ppm; and colored pages of magazines 2,3400 ppm.[5,10]

ABSORPTION

Very little ingested lead is absorbed from the gastro intestinal tract. It is probable that not more than 5 to 10 percent of ingested lead is absorbed from the gut.[15] In

animals, the amount of lead absorbed from the gut appears to be less than in man. Sheep and rabbits, for example, take up less than 1.5% of administered lead over a range of 2-108 mg Pb/day.[14] Zook maintains that young dogs retain 36% of their dietary lead, whereas adults retain only 10 per cent.[2] Several months of ingestion of diets containing 100 ppm of lead and doses as low as 0.3 mg/kg/day have been toxic to immature dogs.[2]

DISTRIBUTION

A large portion of absorbed lead is retained initially in soft tissues as diphosphate and later in bone as insoluble triphosphate. Following acute toxicity lead is easily detected in soft tissues; however, deposition in the bone requires chronic exposure to lead source. Of the retained lead, 60% is located in bone; 25% in the liver; 4% in the kidney; 3% in the intestinal wall; 3% in the reticuloendothelial system; and 5% in other organs.[1,10]

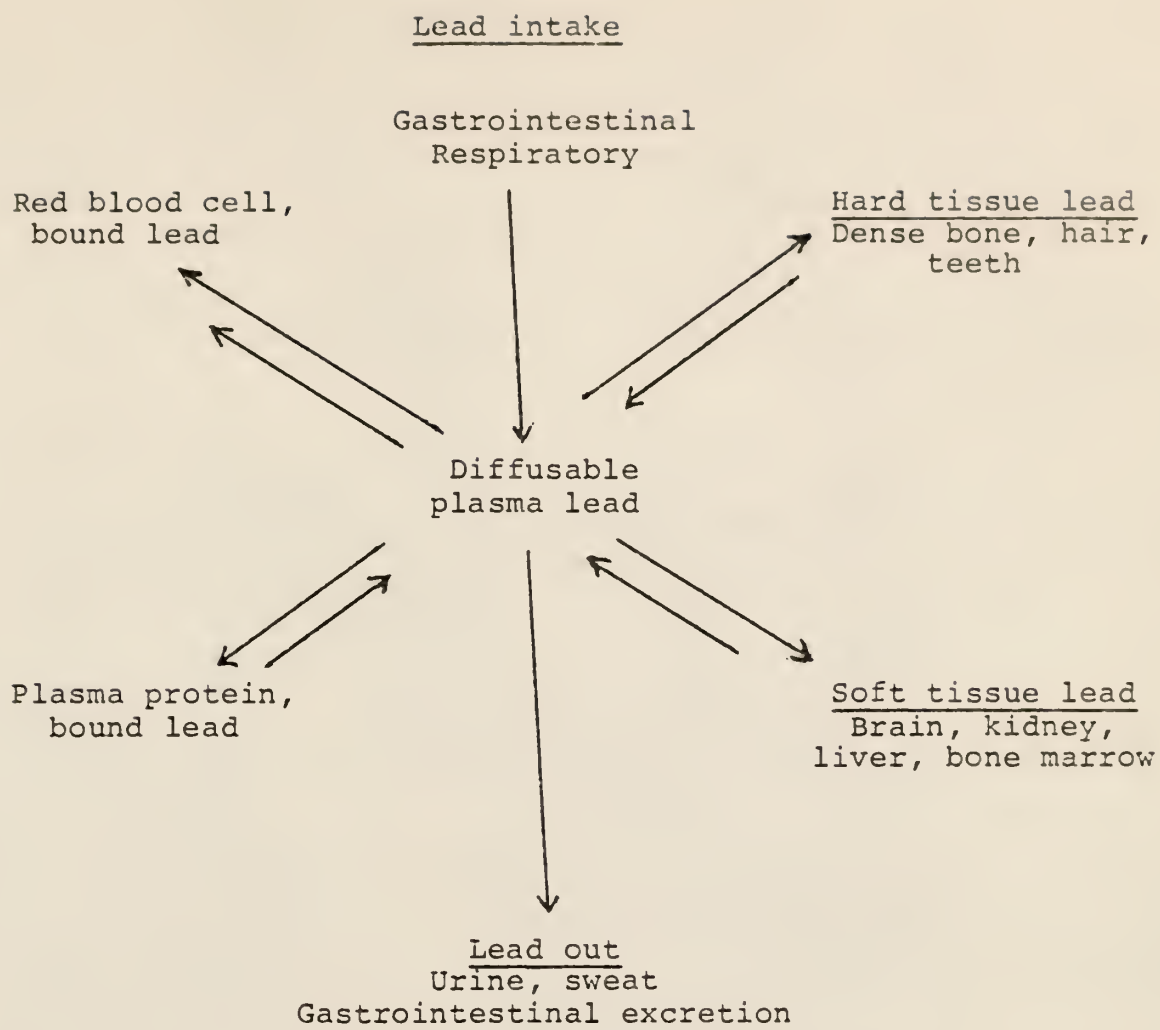

Fig. 1. Schematic view of lead metabolism.

EXCRETION

Lead is slowly removed from the body, primarily by the kidneys. Biliary and milk excretion also occurs.[1] In mice more lead is apparently retained in the body and less excreted in urine and faeces at high environmental temperatures.[4,10]

TOXICITY

The form in which lead is administered does not appear to affect its absorption. Calves, poisoned with a series of lead salts, showed little difference in uptake when the lead was given as the acetate, phosphate, oxide or carbonate or even as dried paint flakes. Table 1 shows toxicity of various salts and the routes.

Table 1

Relative toxicity of lead and its compounds

	Method of introduction		
Order of toxicity	Injection	Ingestion	Inhalation
Most toxic	Lead arsenate	Lead arsenate Lead carbonate Lead monoxide Lead sulphate	Lead carbonate Lead monoxide
Of similar but a lower degree of toxicity	Metallic lead Lead carbonate Lead chromate Lead monoxide Red lead Lead dioxide Lead phosphate Lead sulphate Lead sulphide	Metallic lead Lead chromate Red lead Lead dioxide Lead phosphate Lead sulphide	Metallic lead Lead arsenate Lead chromate Red lead Lead dioxide Lead phosphate Lead silicate Lead sulphide

From Fairhall and Sayers (1940)

(Cited by Waldron and Stofer, 1974)

CLINICAL SIGNS

Two kinds of clinical signs occur in dogs - gastrointestinal and nervous. Although either may occur separately, most cases show both signs. Usually gastrointestinal signs precede nervous signs. Anorexia and vomiting appear first.[1,2,4] Colic, apparently due to smooth-muscle spasms induced by lead[4] is evident by restlessness, whining, groaning and tender abdomen. Constipation has been recorded.[1] Gastrointestinal signs have been seen in about 90% of the cases.[2]

The severity of nervous signs increase as exposure to lead continues. Initial signs may be observed as twitching during sleep. Later periodic convulsions and fits are quite common with continued exposure, and epilepsy is often diagnosed. Hysteria is a consistent manifestation. 90-95% of dogs with hysteria have lead poisoning.[2] Other signs of brain or nerve injury include: nervousness, whining, behavioral changes, retraction of the eyeball with protrusion of the nictating membrane, photophobia, miosis, incoordination, paresis, stupor, esophageal paralysis and apparent blindness.[2,4]

Fever, respiratory embarrassment and conjunctivitis, although occasionally reported, may be due to concommitant disease.[1,4] Dogs exposed to chronic lead intoxication show a marked weight loss, and this may be due to malabsorption of nutrients from gastrointestinal tract, the latter being inflamed.

Differential diagnosis based on history and clinical signs includes heavy metal poisoning, intestinal parasitism, intussusception, canine hepatitis, acute pancreatitis, heat stroke, encephalitis, rabies and most commonly, canine distemper.[1,2,10]

MECHANISM OF ACTION

Lead causes anorexia, colic, loss of weight by its severe astringent action on the mucosa of gastrointestinal tract, leading to gastroenteritis; hence subsequent malabsorption of nutrients and gradual emaciation. Beliles[14] suggests that weakness of extensor muscles, as well as the gastrointestinal signs, may be related to peripheral neuropathy and not to a direct effect on the intestinal mucosa. Seizures are as a result of direct toxic effect on the motor neurons of the brain.

Anemia is as a result of hemolysis of circulating erythrocytes and defective hemoglobin synthesis in the bone marrow. At least three enzymes involved in hemoglobin synthesis are inhibited by lead.[15] Lead affects these enzymes by combining with their sulfhydril (-SH) groups.

CLINICAL DIAGNOSIS

Like any other poisoning, a diagnosis of lead intoxication is dependent on anamnesis of lead exposure to the animal. History of the owner having seen the animal licking painted walls or chewing batteries should be helpful.

Clinical signs described above should form a tentative diagnosis, pending laboratory diagnosis.

Hematologic findings in dogs with lead poisonings are so consistent that they are almost pathognomic. Typical blood changes precede clinical signs except in rare, acute poisonings. Because lead interferes with hemoglobin synthesis, the rubricyte nucleus is retained in the bone marrow for a long time, resulting in variable number of nucleated red blood cells (metarubricytes) in peripheral blood. Basophilic stippling of erythrocytes is almost a constant feature of the disease. Stippled red blood cells (rbc's) contain clusters of very fine blue dots or large blue-black granules, best seen in unfixed direct smears stained with neutral Wright-Giemsa stain.[2] Prolonged exposure to anticoagulants - ethylene-disodium diamine tetraacetic acid (EDTA) may decrease the number of stippled cells by 25 to 30%. The stippling effect occurs when the blood film is prepared and consists of clumped ribosomes.[7] Stippled rbc's are altered immature rbc's which would be reticulocytes if stained supravitally. Basophilic stippling may be seen in some dogs with marked and prolonged anaemia, but rarely to the same degree as in lead poisoning.[3] Although stippling is good evidence for lead poisoning, a diagnosis of lead poisoning cannot be ruled out when stippling does not appear.[1] Other asscciated findings are absolute neutrophilia with a left shift, eosinopenia and monopenia.[4]

Blood lead analysis is the best single antemortem test[8] to confirm lead toxicity, although blood levels and the severity of signs are not related.[4] Assessment of lead in hair or urine is seldom of diagnostic value. Analysis of lead in liver is the most reliable test to diagnose lead poisoning.[8] The concentration of blood lead level in normal dogs ranges from 0.1 to 0.5 ppm. Levels above 0.6 ppm are diagnostic of lead poisoning, but poisoning may be associated with levels in the upper normal range[1], especially with sporadic or past absorption of lead.[7] Normal lead concentration in liver ranges up to 3.5 ppm on a wet weight basis. Values of 5 to 10 ppm are suspicious of lead poisoning and greater than 10 ppm constitutes a definite diagnosis.[2] Lead levels in the kidney greater than 10 ppm are considered diagnostic.[7]

Radiopaque ingested lead substances may be visible in radiographs but must be distinguished from gravel or bone chips.[2,4] Lead lines may be visible in the metaphyses of long bones, particularly radius, ulna and metacorpals. These metaphyseal scleroses develop in immature dogs poisoned over 10 days and appear as dense white bands approximately 2 to 4 mm thick just proximal to the epiphyseal plate.[2,4,9,10] More active growth plates show more pronounced effects of lead poisoning. Lead deposited during endochondral ossification adds little or nothing to the radiopacity.[9] The change is the result of incorporation of lead into sites of active bone formation which results in a dense zone of mineralized cartilage and bone.[2]

PATHOLOGY

Gross changes at autopsy are generally non-specific. Particles of lead paint or other lead substances may be found in the gastrointestinal tract. Gray-black staining of mucosa of the large intestine may result from precipitation of lead sulfide. Longitudinally split long bones may contain white bands at the metaphysis, although radiographs may disclose these sclerotic areas even when they are grossly inapparent.[2,10]

Histologic changes may help in diagnosis. Chromatin marginates and nuclei enlarge, followed by appearance of eosinophilic, acid-fast, intranuclear inclusion bodies in renal and hepatic epithelium. Histologically, sclerotic metaphyseal areas consist of increased numbers and thickness of metaphyseal trabeculae containing calcified (and leadified) cartilagenous cores, which result from the inability of osteoclasts to resorb altred cartilage. Osteoclasts hypertrophy and proliferate. Unaffected osteoblasts continue to lay down new bone on the persisting trabeculae. Vitamin D, phosphorus or bismuth intoxication may be confused radiographically and grossly with these lesions. Lead replaces calcium in the apatite crystal and supposedly responds to parathomone.[2]

Lead encephalopathy is characterized by vascular damage, hemorrhages, edema and areas of neuronal necrosis. In dogs cerebrospinal fluid pressure is usually normal and the brain is not swollen grossly. Other lesions include excess

hemosiderosis and erythrophagocytosis, bone marrow hyperplasia and extramedullary hematopoiesis, pancreatitis and pancreatic fibrosis, necrosis of striated muscle fibers, and decreased fertility or sterility.[2,10]

TREATMENT

Therapy of lead poisoning is designed to prevent further absorption by removing lead from gastrointestinal tract, the blood, and body tissues rapidly and to alleviate marked neurological signs. Lead should be removed from intestinal tract before chelation therapy, as chelating agents may enhance absorption of lead.[4] Gastrointestinal tract should be cleaned by the use of enemas, laxatives, emetics or surgery may be required.[7]

Chelating agents remove lead from the body by forming non-toxic compounds that are readily removed by the kidney.[2] Currently the chelating agent of choice is calcium disodium ethylenediaminetetracetate (CaEDTA) usually administered at the rate of 10 mg/lb four times daily for 4 to 5 days.[12] Daily dose should not exceed 1 g nor should therapy be continued for more than 5 consecutive days. Side effects of CaEDTA have been reported in dogs and these include: depression, anorexia, vomiting and diarrhea. The drug depresses turnover of DNA and effects normal regrowth of epithelial cells of intestinal vili.[2] The main action of CaEDTA is to remove lead from bony tissues, not from soft tissues. Too rapid mobilization can cause signs of acute lead

poisoning.[1] CaEDTA is generally administered IV in 5% dextrose or SQ at a concentration of no more than 10 mg CaEDTA per ml solution. Significant improvement usually occurs in 36 to 48 hours.[7]

Barbiturates or tranquilizers may be used to control convulsions. Mannitol or dexatmethazone may be used to relieve cerebral edema. Supportive therapy should be provided as required.[2] Prognosis is generally good and depends on the promptness of treatment and degree of neurological involvement. If therapy is initiated immediately, recovery is rapid in 95% of the cases involved.[10,12]

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A LITERATURE REVIEW OF EFFECT OF LEAD IN BONE MARROW

HEMATOPOIESIS

After birth, erythropoiesis, granulopoiesis, and megakarypoiesis occur principally in the bone marrow. At first, the marrow of all bones is active in blood-cell production since there is more demand for replacement of fetal erythrocytes with red blood cells more suited to serve the air-breathing mammal. As demand for erythrocytes decreases with approaching maturity, hematopoiesis recedes from the shafts of long bones.[1] Red, hematopoietically active marrow is replaced by resting yellow marrow. Active hematopoiesis continues throughout life in all flat bones, such as the sternum, ribs, pelvis, vertebrae, and skull, as well as the epiphyses of long bones.[1,3,8] Hematopoiesis takes place in the space between the vascular sinuses of the bone marrow.[1]

The yellow marrow is limited to three types of cells: reticular cells, which are connected with the endosteum and blood capillaries; the endothelium forming the walls of capillaries and sinusoids; and the fat cells. The fat cells act to occupy space as hematopoiesis recedes and to give up space as demand for expansion of red marrow occurs in response to continuous blood loss or hemolytic anemia. Transition of yellow to red marrow is influenced by the hormone erythropoietin.

Blood supply of the marrow consists of the arterial system that runs longitudinally through the axis of the bone

and gives off radial branches that terminate at the periphery of the marrow to form vascular sinuses that carry the blood back to central vein.[1] The sinusoidal microcirculation is designed specifically to serve hematopoiesis, and when it is irreversibly injured, as demonstrated in locally irradiated bone marrow of the rat[2], hematopoietic regeneration initially took place 2 to 6 months later. Aplasia developed as the sinusoidal microcirculation became disrupted by chronic changes in bone marrow structure.

THE STEM CELL

To continue production of blood cells, it is postulated that a compartment of primitive stem cell exists in the bone marrow that initiates production of additional precursor cells for the specific cell line that requires expansion.[1]

The origin of erythrocytes and all leucocyte types from a pluripotential stem cell has gained acceptance as the most logical explanation of events in cell replacement following experimental destruction of hematopoietic cells by irradiation.[4,5,6] The demonstration of Philadelphia chromosome designated (Ph^1) by Whang[7] in erythroid, granulocytic and possibly megakaryocytic cells of leukemic patients further implicates a common precursor cell for each of the three cell lines. The stem cell is considered a primitive cell of mesenchymal origin existing in the bone marrow and other hemopoietic tissues. Its presence has been demonstrated

in circulating blood.[9,10,11] Lewis[12] observed the stem cell content of bone marrow cells to be 50 to 150 times that of normal blood leucocytes in mice, and Loutit[13] postulated a stem cell concentration of 1:1,000 cells in bone marrow and 1:100,000 leucocytes in blood. It is not known how the stem cell gets into bone marrow; erythropoietin has been incriminated. Fliedner[14] administered radiolabeled triated thymidine (^{3}HTdR) to pregnant rats every 6 hours from the ninth day of pregnancy to paturition. Autoradiographs indicated that all cells of newborn rats were heavily labelled. A significant number of cells which were neither leucocytic nor erythrocytic in origin were also labelled for weeks. He further administered some additional ^{3}HTdR to newborn rats every 8 hours for 6 weeks.[15] He treated one group with hydroxyurea to produce hypoplasia of the bone marrow. These rats were sacrificed at intervals, a rapid recovery of hematopoiesis followed in 2 to 3 days after the last dose of hydroxyurea, with full recovery within two weeks. The label appeared in marrow lymphocytes, blast cells, and early precursors of hematopoietic cells. These studies apparently support the hypothesis that the stem cell is a small lymphocytelike cell that on demand can give rise to granulocytic, megakaryocytic and erythrocytic precursor cells.

It is thought that the primitive stem cell becomes a 'committed hematopoietic precursor cell' and this may be under the influence of erythropoietin hormone which is produced in

the kidney glomerular epithelium. Anoxia is thought to be the stimulus of erythropoiesis. Committed stem cell differentiates into recognizable hematopoietic cells by specific poietins of which erythropoietin is the best known.[16,17] Thus, different cell series become differentiated like rubriblasts leading to increased erythrocytes, myeloblasts leading to increased leucocytes, and megakaryoblasts leading to increased megakaryocytes. There are two models of stem cells:multipotential stem cells are capable of differentiating into the various cell series of the bone marrow; unipotential stem cell theory proclaims individual stem cells for the various cells of the hemopoietic system. Multipotential stem cell is the most accepted way by which blood cells originate.[7,14]

BONE MARROW CELLS

Erythroid Series.

Rubriblast. This is a round large cell presenting a narrow rim of dark blue cytoplasm. The nucleus occupies most of the cell, is usually centrally located but may be eccentric. The chromatin is finely stippled and of a reddish tinge. Nucleoli and nucleolar rings are present.[1]

Prorubricyte. Similar in appearance to the rubriblast, with the exception that the nuclear chromatin material may be less condensed, and nucleoli or rings are absent.[1]

Rubricyte. This cell is smaller than the prorubricyte,

and still retains a narrow rim of deep blue cytoplasm. The nuclear chromatin is condensed and separated by light streaks giving the so-called cartwheel appearance.[1] In late stage synthesis of hemoglobin is well under way and this produces a change in the color of cytoplasm to light blue or gray. Condensation of nuclear material progresses fast so that the appearance is one of dark blobs separated by light streaks. In the cat and horse normochromic rubricyte nucleus remains viable, while the cytoplasm stains similarly to the mature erythrocyte.

Metarubricyte. The stage is depicted by the presence of a nonviable nucleus. The nucleus is solidly black or pyknotic. It may be fragmented, partially extruded, or partially autolyzed. The cytoplasm may be polychromatic or normochromatic, depending on the extent of hemoglobin synthesis.[1]

Reticulocyte. This is non-nucleated erythrocyte that, when stained with new methylene blue, presents one or more granules or a diffuse network of fibrils. With Romanowsky stains, the reticulocyte is commonly polychromatophilic and infrequently may contain an eccentrically placed nuclear remnant called Howell-Jolly body.

Erythrocyte. Non-nucleated definitive cell of the series with no mitochondrial material left.

Myeloid Series

Myeloblast. This is a large, round to irregular cell in

shape, with finely stippled chromatin containing one or more nucleoli or nucleolar rings.[1] The cytoplasm is blue (basophilic) but generally lighter in color than that of the rubriblast. There are no specific granules in the cytoplasm.

Progranulocyte (Promyelocyte). Same size as myeloblast, and its nuclear chromatin is finely stippled. The cytoplasm is basophilic and commonly presents reddish azurophilic granules or primary granules. There are no specific granules.

Myelocyte, neutrophilic. This is smaller than the progranulocyte, with beginning condensation of the nuclear chromatin. The cytoplasm is light blue and contains few to numerous neutrophilic granules.

Myelocyte, eosinophilic. This is generally larger than the neutrophilic myelocyte. The cytoplasm is more basic,[1] and the granules are distinctly orange.

Myelocyte, basophilic. This is a very rare cell in the bone marrow preparations. In the cat, the granules are of two types: numerous small, round, and pinkish and fewer large, round and black. In other animals, the granules are metachromatic or black.[1]

Metamyelocyte (neutrophilic, eosinophilic and basophilic). The nucleus is indented to assume a kidney-bean shape. This cell is no longer capable of division. The cytoplasm may retain a slight basophilia in the neutrophil but the other two forms retain a bluish cytoplasm as a characteristic of the mature cell of the series. The specific granules identify

the cell as neutrophilic, eosinophilic, or basophilic.

Band form. The nucleus has parallel sides but twists to conform to the space within the cytoplasm. The shape of the nucleus is commonly seen as horseshoe or S-form. The nuclear membrane is smooth and any irregularity of the membrane or indentation indicates maturity has been attained, hence requiring classification as a segmented and not a band cell.[1]

Segmented neutrophil (Polymorphonuclear leucocyte). The nucleus usually has several lobes separated by filaments, however, monolobed nucleus is also seen. In some species this cell is called a heterophil.

Eosinophil. The cytoplasm contains eosinophilic granules. Granules vary in size, shape, and number within the species of the animal. The granules are eosinophilic but vary in intensity of reddish color between species. The cytoplasm is usually light blue.

Basophil. The cytoplasm is basophilic with few to many metachromatic granules, depending on the species. Granules are round and pinkish in the cat, although some mature basophils may retain a few small darker-staining granules.

Maturation of the Megakaryocyte

Megakaryoblast. The first recognizable cell of this series is larger than other blast cells of bone marrow. It has two reddish nuclei with a distinct chromatin pattern. The cytoplasm is small in amount and takes a deep blue stain.

Promegakaryocyte. Maturation leads to multiplication

of nuclei by mitotic division without division of the cytoplasm (endocytosis). Number of nuclei may be 4, 8, 16 or 32. In the larger cells, the nuclei are commonly fused into a single irregular mass. The cytoplasm retains the deep blue color of the blast form and is limited to a relatively narrow rim around the nuclear mass.

Megakaryocyte. The cytoplasm is pale and presents pinkish azurophilic granules. The cytoplasm is increased considerably over that of the promegakaryocyte. The formation of granules begins in the perinuclear zone and gradually extends to the periphery of the cell. Cell size is variable and depends upon the number of nuclear divisions that had taken place before DNA synthesis was terminated by the beginning maturation of the cytoplasm. In films of aspirated bone marrow, some megakaryocytes may present pseudopodlike extensions and a nuclear mass without cytoplasm, or cytoplasm without a nucleus may be encountered.

Thrombocytes or platelets. Individual platelets are cytoplasmic structures of megakaryocytes. They vary in shape and in size and contain reddish granules in a light blue field. In blood, they are often commonly clumped.

Other Cells

There are other cells usually not classified. These include osteoclasts, mitotic figures, hematogones which are round, pyknotic nuclei extruded from metarubricytes, lymphocytes especially small type, monocytes, macrophages, plasma cells, reticuloendothelial (RE) nuclei which are

pinkish-staining roundish structures containing one or more blue nucleoli, unclassified cells, and degenerated cells.

EFFECTS OF LEAD ON CIRCULATING RED CELLS

Osmotic Fragility

A great deal of the work on the pathogenesis of the anemia of lead poisoning has been carried out by many workers.[1,18,19,20] These authors[18] found that normal washed red cells became more resistant to hypotomic saline after exposure to lead chloride solution. Cells that had been exposed to solutions of lead of concentrations as high as one part per 100,000 did not hemolyse in 0.1% saline whereas normal cells were completely hemolysed in 0.25% saline. These observations on the effects of lead on the osmotic fragility of the red cell have been almost unanimously corroborated. Indeed the only dissenting voice seems to be that of Scroczyriski (cited by Waldron) who induced chronic and acute lead poisoning in rabbits by injecting a 0.9% solution of alkaline lead acetate into the marginal vein of the ear and found that red cells from both groups of animals showed a marked decrease in resistance to hypotonic saline solutions. The resistance was markedly decreased in the acute group than in the chronic group. Many authors, however, have recorded a normal or increased resistance to hypotonic saline solutions.

The mechanism by which lead causes osmotic changes in red cells is not as yet understood. Lead affects the cation permeability of the cell membrane resulting in increased

potassium (K^+) loss.[18] At concentrations of 100 uM/L, potassium loss is much increased, the effect decreasing with time. It is thought that increased osmotic resistance may indicate that more water could enter the cells before a critical spheroidal form is reached and lysis occurs. No recent literature has been reported on osmotic fragility.

Mechanical Fragility

Although Aub et al. (cited by Waldron) found that the osmotic fragility of the red cells exposed to lead decreased, they found, by contrast, that the mechanical fragility was much increased. From his observations, Aub et al. concluded that red cells exposed to lead were less able to withstand the dynamic trauma consequent upon passage through the capillaries and so broke up more readily than normal cells, giving rise to the anaemia of lead poisoning. An increase in mechanical fragility was reported in cases of human plumbism.[18] It was found that mechanical fragility index of red cells was markedly increased on standing[18], and the disparity between their results and those of the earlier workers would be explained if the blood used by older workers was not fresh, i.e. had stood for more than one hour. In four of the 10 cases reported, incubation caused a more than normal increase in mechanical fragility[18] (16.5%, 19.7%, 22.2%, and 26% compared with a mean control of 10.5 $\pm$ 3.5%).

The mechanism by which lead causes an increased mechanical fragility is not well understood. It has been reported that lead decreases half life time of erythrocytes[18,20],

and this may document for hemolysis from increased mechanical fragility. No recent literature has been reported on this subject. Mitema[21] and Oehme (submitted data) have recently investigated if lead has any effect on glutathione activity. They observed no significant changes in activity of the enzyme in lead dosed animals compared to control animals. Gluthathione is a tripeptide, with sulfhydryl (-SH) group compound, which is thought to protect red blood cell membrane and integrity.

Red Cell Survival

Relatively few studies on the red cell survival time in experimental or human lead poisoning have been made. The most comprehensive study has reported, significantly lower values than normal were obtained using both radio-chromium tagging.[18] It has been reported that cells removed from two of four patients with lead poisoning had a significantly shorter half-life ($T_{\frac{1}{2}}$), 20 days than the normal, 27 to 34 days.[18] After treatment with ethylene diaminetetra acetic acid (EDTA) the $T_{\frac{1}{2}}$ returned to normal. Leikin and Eng[19] reported erythrocyte survival times in seven children with lead poisoning. The $T_{\frac{1}{2}}$ was reduced in five of the seven patients, the shortest survival times being seen in those children with the shortest exposure to lead. Although there seems no doubt that in some cases of lead poisoning, a shortened red cell life span is found. It is reported that anemia may not result until the life span is less than 40 days. Above this value compensatory erythropoiesis is

sufficient to prevent anemia developing. The mechanism by which lead produces a shortened life span in red cells is not clear, but it seems likely to be an effect on the developing red cells in the bone marrow, where the concentration of lead is higher than in the peripheral blood.

Stippled Cells

These are invariable but by no means constant accompaniment of lead poisoning.[18-20,22-24] The enumeration of the number of stippled cells in the peripheral blood has been advocated as a means of controlling exposure to lead, but nowadays, stippling is generally regarded as an unreliable index of lead absorption. The degree of stippling does not correlate well with the intensity of exposure and is found in a wide variety of other hematological disorders, like hemolytic anaemias, thalassaemia, leukemia, and after exposure to aniline, arsenic, benzene, carbon monoxide and copper. Stippled cells origin is not well understood, however, they are thought to be degenerative or regenerative changes occurring in the ribosomes[18,22] of red blood cells. These cells can be seen both in peripheral blood smears and bone marrow preparation smears. Another study concluded that the stipple material is composed exclusively of ribonucleoproteins.[18] Electron microscopic studies showed that the stipple material might be aggregates of ribonucleic acid (RNA) around the mitochondria.[18]

HEMOGLOBIN SYNTHESIS

The mechanism of porphyrin synthesis is now well-known and a number of comprehensive surveys have been devoted to this study.[23,25-29] Studies with ^{15}N- and ^{14}C-labeled glycine have shown that glycine[23] provides the nitrogen atoms and eight carbon atoms, whilst succinyl CoA supplies the remaining 26 carbon atoms for synthesis of protoporphyrin molecule. 'Activated' glycine and 'activated' succinic acid (succinyl CoA) react to form delta-amino-levulinic acid (ALA). The delta-ALA is synthesized only in mitochondria of red blood cells, and ALA synthetase is the rate-limiting enzyme in the entire sequence. Pyridoxal phosphate (vitamin B_6) is necessary for the activation of glycine required for its initial condensation with succinyl CoA; an abnormality in the metabolism of this enzyme results in hypochronic anaemia correctable only with Vitamin B_6. Pantothenic acid is a component of coenzyme A, hence it is required for the formation of delta-ALA. Its deficiency is known to produce hypochromic anaemia in birds and animals, but not in man. Two molecules of delta-ALA react to form a substituted pyrrole, porphobilinogen (PBG). Four molecules of PBG react, ultimately forming the tetrapyrrolic ring component, uroporphyrinogen III. Both uroporphyrinogen I synthetase and uroporphyrinogen co-synthetase are required for the formation of uroporphyrinogen III. Uroporphyrinogen III is converted to coproporphyrinogen III and then to protoporphyrinogen, which, upon oxidation,

yields protoporphyrin III. Protoporphyrin III subsequently combines with four moles of iron to form heme, four molecules of which finally conjugate with four globin molecules to form hemoglobin molecule.

EFFECT OF LEAD ON HEME SYNTHESIS

The effect of lead on heme synthesis is shown in Fig. 1. At least three steps in heme synthesis may be affected by lead. Delta-ALA dehydratase (ALA-D) is probably the enzyme in the heme pathway that is most sensitive to lead. Inhibition of this enzyme results in a block in utilization of delta-ALA and in subsequent decline in heme synthesis.[30] Granick and Levere[31] have proposed a negative feedback control of heme synthesis in which delta-ALA synthetase activity is repressed, resulting in increased activity of the enzyme and increased synthesis of delta-ALA. Hence rise of delta-ALA in urine and blood. A third abnormality of heme synthesis in lead intoxication is inhibition of the enzyme ferrocheletase (heme synthetase).[32] Ferrochelatase catalyzes the incorporation of the ferrous ion into the porphyrin ring structure. Hence inhibition of the enzyme may lead to accumulation of iron in the form of apoferitin and ferruginous micelles in mitochondria of bone marrow reticulocytes of lead poisoned animals.[22]

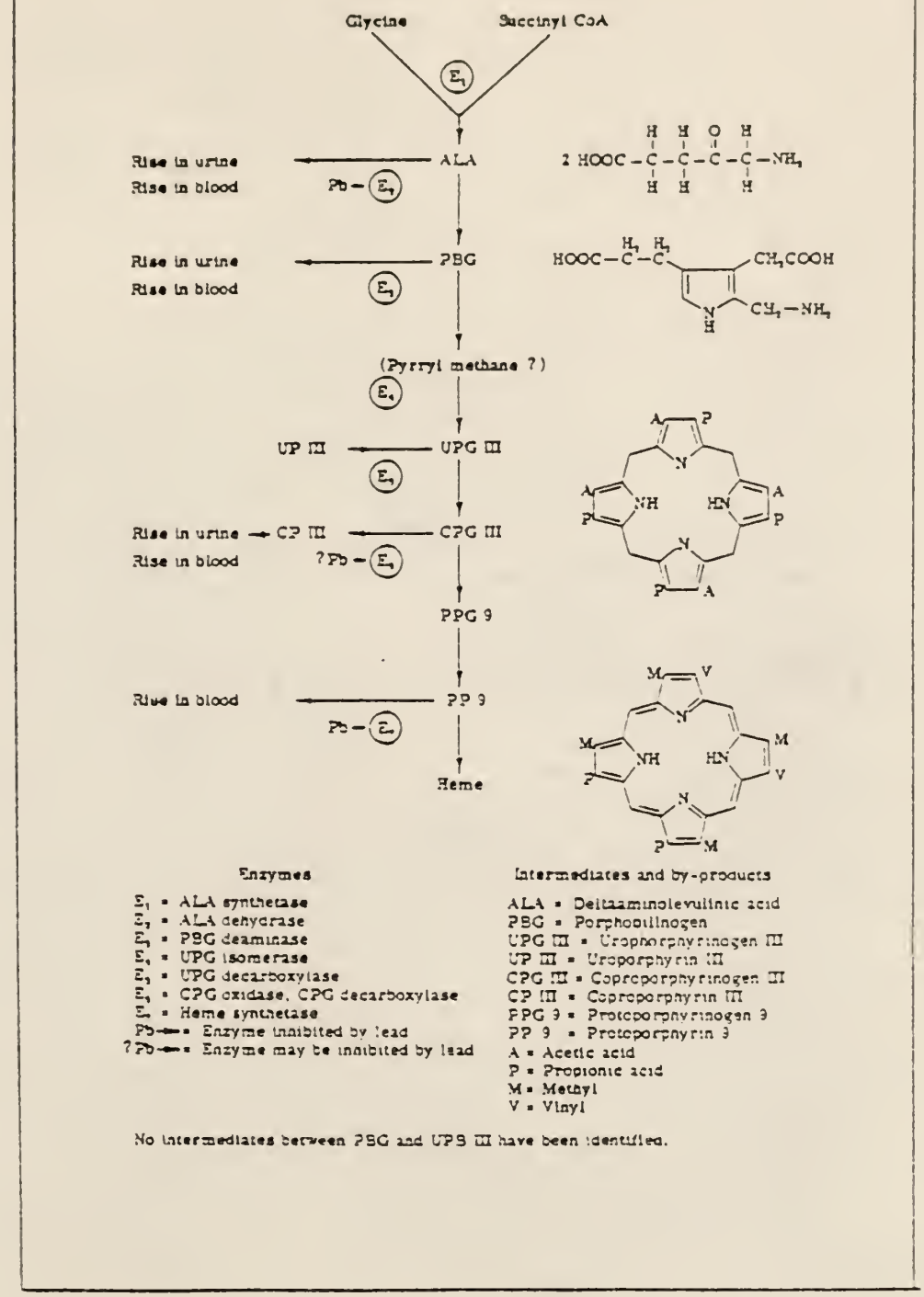

Fig 1. The synthesis of heme showing sites inhibited by lead.

STAINABLE IRON GRANULES IN LEAD POISONING

In cases of chronic lead poisoning it has been seen that Prussian blue (PB) reaction is seen in the early erythroid series and macrophages in the bone marrow.[33-35] Developing erythroid cells which contain stainable iron granules are known as 'sideroblasts'. These cells are found normally, but they are absent, or their number is greatly reduced in patients with iron deficiency anemia. Iron granules are usually seen in hemolytic anemias[22] in the form of ferritin or hemosiderin.

Ferritin is a complex macromolecule with protein subunits that form around an inorganic core of iron oxyhydroxide abbreviated as $(Fe\ OOH)_X$. The core also contains phosphate up to one-eighth its own mass. In fully loaded molecule of ferritin, core iron constitutes approximately 26% of the dry molecule mass. Hence, a single molecule of ferritin may contain 400 atoms of iron.[33] By contrast, natural apoferritin (the protein moeity) contains only traces of iron. Ferritin has a diameter of 120^{o}A and is isometric. It can be synthesized by many cells - hepatocytes, erythroblasts, fibroblasts, columnar absorbing cells and heart muscle cells.

Hemosiderin has been used to denote iron-positive granules which after application of Potassium ferrocyanide ($K_4Fe(CN)_6$) and hydrochloric acid gives Prussian (Berlin) blue reaction (hereafter referred to as PB reaction). It is clear that PB-positive material, denoted by the term "hemosiderin",

includes both water-insoluble material that is currently termed "hemosiderin" by biochemists as well as ferritin.[33] Hemosiderin is chemically heterogenous, the common feature present being hydrous ferric oxide or oxides, which are brown and give PB-reaction. Structure of hemosiderin is not well understood by electron microscopists.

Nearly fifty times as much lead is found in bone marrow as peripheral blood[18]; it is therefore logical that lead affects "ferro-chelatase" enzyme[32] which catalyzes incorporation of iron into purphyrin ring during hemoglobin synthesis. Hence inhibition of this enzyme may lead to accumulation of iron in the form of ferritin and hemosiderin which give PB-reaction. Ritcher[33] has reported that ferritin or hemosiderin vanishes within 4 days if no additional lead is given.

SUMMARY OF EFFECTS OF LEAD ON BONE MARROW CELLS

It is reported that lead may cause erythroid hypoplasia in the marrow. Lead interferes with at least three enzymes in mitochondria and cytoplasm of erythroid cells involved in hemoglobin synthesis in bone marrow. Positive PB-reaction due to iron granules has been seen in marrow smears of lead poisoned patients.

No literature has reported any direct effect of lead on myeloid or erythroid cells of the canine bone marrow. In our study we observed increases of the myeloid:erythroid (M:E) ratios, segmented neutrophils, myeloid series cells, and iron

granules in a chronic exposure of lead (5 mg Pb Kg/b wt) on dogs.

Lead may affect early erythroid cells by interfering with the enzymes involved in hemoglobin synthesis, thus arresting maturation of the cells and leading to erythropathy. It has been reported that lead causes a shortened life span of erythrocytes; it is possible that some of the early forms of erythroid series may be affected as well. This may document for erythroid hypoplasia. It is possible that lead may also arrest the release of segmented neutrophils from the marrow explaining for their increased number in the marrows.

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INDIVIDUAL ANIMAL DATA

NOTE: THE JOB XPRS1709 HAS BEEN RUN UNDER RELEASE 76.6B OF SAS AT KANSAS STATE UNIVERSITY.

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1          DATA ONE1
2          INPUT DOG 2.  DAY 3.  GRP 1.  TMT 1.  RBC 3.  WBC 3.  MBLST 2.  PMBLST 2.
3          METAN 2.  METAB 2.  METAE 2.  BANDN 2.  BANDB 2.  BANDE 2.  SEGN 3.
4          SEGB 2.  SEGE 2.  RBLST 2.  PRORCYT 2.  RCYTE 3.  MRCYTE 3.  MONO 2.
5          UNDIF 2.  ABS 3.2  PCV 2.  GMHB 3.1  LEAC 4.1 ;
6          RBC = RBC * 10 ;
7          WBC = WBC * 100;
8          MSERIS = SUM (MBLST,PMBLST,METAN,METAB,METAE,BANDN,BANDB,BANDE,SEGN,
9               SEGB,SEGE);
10         ESERIS = SUM ( RBLST,PRORCYT,RCYTE,MRCYTE );
11         MF = MSERIS / ESERIS ;
12         ABS1 = 310.4 * ABS;
13         GSH1 = ABS1 / PCV;
14         GSH2 = ABS1 / GMHB;
15         *DOG=DOG NUMBER
16         *DAY=DAY OF EXPERIMENT
17         *GRP GROUP 1=CONTROL 2=2MG LEAD/KG/DAY 3=5MG LEAD/KG/DAY ORALLY
18         *TMT TREATMENT  0=NO LEAD GIVEN 1=LEAD GIVEN 2=EDTA GIVEN RBC=RBC IN THOUSANDS
19         *WBC=WBC IN THOUSANDS MBLST=NO OF MYELOBLASTS  PMBLST=NO OF PROMYELOCYTES
20         METAN=NO OF NEUTROPHIL MYELOCYTE METAB=NO OF BASO MYELOCYTES
21         *METAE=NO OF EOSIN MYELOCYTES  BANDN=NO OF BAND NEUTROP BANDB=NO OF BAND BASO
22         *BANDE=NO OF  BAND EOSINO  SEGN=NO OF SEG NEUTROPHILS SEGB=NO OF SEG BASO
23         *SEGE=NO OF SEG EOSINO RBLST=NO OF RUBRIBLASTS PRORCYT=NO OF PRORUBRICYTES
24         *RCYTE=NO OF RUBRICYTES MRCYTE=NO OF METARUBRICYTES
25         *MONO=NO OF MONONUCLEAR LEUCOCYTES UNDIF=NO OF UNCLASSIFIED CELLS
26         *ABS=ABSORBANCE AT 412 NM USING SPECTROPHOTO  GSH1=MG GLUTATHION/100ML RBC
27         *GSH2=MG GLUTATHION/GM HB PCV=%PACKED CELL VOLUME
28         *GMHB=%HEMOGLOBIN LEAD=BLOOD LEAD CONC UG/100ML;
29         CARDS;

NOTE: MISSING VALUES WERE GENERATED AS A RESULT OF PERFORMING
      AN OPERATION ON MISSING VALUES.
      EACH PLACE IS GIVEN BY: (NUMBER OF TIMES) AT (LINE#):(COLUMN#).

      90 AT 6:7   90 AT 7:7   92 AT 8:10   92 AT 10:10   92 AT 11:6

NOTE: DATA SET WORK.ONE HAS 190 OBSERVATIONS AND 33 VARIABLES. 48 OBS/TRK.
NOTE: THE DATA STATEMENT USED 1.53 SECONDS AND 102K.

220        PROC PRINT;
221        TITLE EFFECT OF LEAD ON BONE MARROW AND PERIPHERAL BLOOD PROFILES;

NOTE: THE PROCEDURE PRINT USED 7.56 SECONDS AND 140K AND PRINTED PAGES 1 TO 8.

NOTE: SAS USED 140K MEMORY.

NOTE: BARR, GOODNIGHT, SALL AND HELWIG
      SAS INSTITUTE INC.
      P.O. BOX 10066
      RALEIGH, N.C. 27605
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EFFECT OF LEAD ON BONE MARROW AND PERIPHERAL BLOOD PROFILES

14:36 WEDNESDAY, AUGUST 9, 1978

OBS	DOG	DAY	GRP	TMT	RBC	WBC	MBLST	PMBLST	METAN	METAB	METAE	BANDN	BANDB	BANDE	SEGN	SEGB	SEGE	MBLST
1	28	0	5	0	4400	19100	12	8	8	2	0	60	0	0	102	0	0	4
2	28	7	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	28	14	5	1	4910	19900	14	34	26	0	0	86	0	0	78	2	0	20
4	28	21	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	28	28	5	1	4940	16600	10	10	18	2	4	40	2	0	142	0	4	8
6	28	35	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	28	42	5	1	4290	12000	20	24	20	0	0	76	0	2	96	0	2	2
8	28	49	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	28	56	5	1	4960	12900	6	30	28	0	0	48	2	0	160	0	0	22
10	28	63	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
11	28	70	5	1	5260	11400	.	.	.	.	.	.	.	.	.	.	.	.
12	28	77	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13	28	84	5	1	4740	15300	12	28	30	0	0	112	0	2	124	0	4	8
14	28	91	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
15	28	98	5	0	5100	12700	22	32	60	0	2	88	0	4	126	0	8	10
16	28	105	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
17	28	112	5	0	5290	10700	6	20	41	0	2	80	0	2	126	0	0	4
18	28	119	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
19	28	126	5	0	6040	12100	12	11	28	0	0	89	0	4	85	0	3	10
20	29	0	2	0	4750	18300	6	8	14	0	0	78	0	0	110	0	0	10
21	29	7	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
22	29	14	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
23	29	21	2	1	4510	12100	12	16	24	2	0	84	4	0	126	2	2	14
24	29	28	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
25	29	35	2	1	5410	9900	18	22	30	0	0	49	0	0	100	0	2	34

OBS	PROMCYT	MCYTE	MRCYTE	MGNO	UNDIF	ABS	PCV	GMHB	LEAD	MSERIS	ESERIS	ME	ABS1	GSH1	GSH2
1	8	62	126	43	20	0.50	33	9.1	7.4	192	200	0.96000	155.200	4.7030	17.0549
2	.	.	.	.	.	0.30	33	9.1	6.0	.	.	.	93.120	2.8218	10.2330
3	18	98	80	52	22	0.65	32	9.7	30.0	240	216	1.11111	201.760	6.3050	20.8000
4	.	.	.	.	.	0.45	32	9.5	30.3	.	.	.	139.680	4.3650	14.7032
5	30	94	86	49	27	0.75	34	10.3	31.2	240	218	1.10092	232.800	6.8471	22.6019
6	.	.	.	.	.	0.60	33	10.0	41.6	.	.	.	186.240	5.6436	18.6240
7	12	80	120	41	19	0.65	32	9.5	51.9	240	214	1.12150	201.760	6.3050	21.2379
8	.	.	.	.	.	0.63	34	10.2	104.0	.	.	.	195.552	5.7515	19.1718
9	28	80	90	50	17	0.30	34	9.9	69.3	274	220	1.24545	93.120	2.7388	9.4061
10	.	.	.	.	.	0.60	34	10.2	73.9	.	.	.	186.240	5.4776	18.2580
11	.	.	.	.	.	0.73	34	10.5	76.6	.	.	.	226.592	6.6645	21.5802
12	.	.	.	.	.	1.25	35	10.5	100.3	.	.	.	388.000	11.0857	36.9524
13	7	88	102	43	28	0.83	34	9.9	96.6	312	205	1.52195	257.632	7.5774	26.0234
14	.	.	.	.	.	0.90	39	11.5	96.4	.	.	.	279.360	7.1631	24.2522
15	22	76	124	50	52	0.87	37	10.7	86.0	342	232	1.47414	270.048	7.2980	25.2381
16	.	.	.	.	.	1.10	37	11.5	76.5	.	.	.	341.440	9.2201	29.6904
17	22	82	112	60	42	0.90	36	11.3	52.0	277	220	1.25909	279.360	7.7600	24.7221
18	.	.	.	.	.	0.85	35	11.3	48.0	.	.	.	263.840	7.5383	23.3467
19	10	89	83	45	32	0.80	39	12.0	50.0	230	200	1.19000	248.320	6.3672	19.4000
20	12	80	90	50	38	0.65	36	10.7	4.8	216	200	1.08000	201.760	5.6044	18.8561
21	.	.	.	.	.	0.40	32	9.7	6.1	.	.	.	124.160	3.8800	12.8000
22	.	.	.	.	.	0.75	31	9.3	15.1	.	.	.	232.800	7.5097	25.0323
23	20	122	70	37	20	0.45	33	10.3	18.0	272	226	1.20354	139.680	4.2327	13.5612
24	.	.	.	.	.	0.75	34	10.9	23.5	.	.	.	232.800	6.8471	21.3578
25	44	152	50	61	34	0.70	36	11.3	33.2	237	200	0.84643	242.112	6.7253	21.4250

EFFECT OF LEAD ON BONE MARROW AND PERIPHERAL BLOOD PROFILES

14:36 WEDNESDAY, AUGUST 5, 1970

OBS	DOG	DAY	GRP	TRT	RBC	WBC	RBLST	PRBLST	METAN	METAB	METAE	BANDN	BANDB	BANDE	SEGN	SEGB	SEGE	RBLST
26	29	42	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
27	29	49	2	1	4550	9700	22	15	49	0	0	41	2	2	110	0	1	30
28	29	56	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
29	29	63	2	1	4560	0800	20	24	24	0	0	76	0	2	160	0	0	20
30	29	70	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
31	29	77	2	1	4970	9500	22	34	54	0	0	50	0	2	126	0	6	26
32	29	84	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
33	29	91	2	1	5490	9900	14	32	68	0	0	104	0	6	176	2	4	14
34	29	98	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
35	29	105	2	2	5260	10300	12	38	36	0	0	112	0	2	152	0	6	14
36	29	112	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
37	29	119	2	2	5300	9600	12	14	25	0	0	00	0	3	100	0	4	12
38	29	126	2	2	5800	10300	8	15	24	0	3	90	0	0	85	0	0	10
39	30	0	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
40	30	7	2	0	4410	13100	2	8	20	2	0	56	2	0	84	0	0	6
41	30	14	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
42	30	21	2	1	5150	16600	24	20	12	0	0	44	2	0	80	4	2	20
43	30	28	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
44	30	35	2	1	5230	15000	18	20	24	0	0	50	0	0	94	0	0	26
45	30	42	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
46	30	49	2	1	4610	11700	20	21	30	0	3	55	1	0	90	2	1	20
47	30	56	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
48	30	63	2	1	4770	12000	12	40	36	0	0	56	0	10	94	0	2	4
49	30	70	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
50	30	77	2	1	5410	10300	22	28	48	0	0	90	0	2	120	0	5	36

OBS	PROMCYT	MCYTE	MMCYTE	MONO	UNDIF	ABS	PCV	MHB	LEAD	MSERIS	ESERIS	ME	ABSI	GSHI	GSH2
26	.	.	.	.	.	0.63	36	11.1	37.7	.	.	.	195.552	5.43200	17.6173
27	40	50	70	48	25	0.43	33	10.2	34.4	242	206	1.17476	133.472	4.04461	13.0855
28	.	.	.	.	.	0.60	35	11.5	49.0	.	.	.	186.240	5.32114	16.1940
29	38	86	100	57	36	0.60	33	10.2	30.1	306	244	1.25410	186.240	5.64364	18.2500
30	.	.	.	.	.	0.63	37	11.7	44.1	.	.	.	195.552	5.20515	16.7130
31	34	84	82	49	40	0.63	37	12.2	44.1	342	226	1.51327	195.552	5.20519	16.0289
32	.	.	.	.	.	0.87	37	11.5	54.4	.	.	.	270.048	7.29855	23.4024
33	22	102	96	41	89	0.95	30	12.0	50.4	356	234	1.52137	294.800	7.76000	24.5133
34	.	.	.	.	.	0.81	30	11.3	57.0	.	.	.	251.424	6.61642	22.2459
35	14	96	80	32	20	1.05	42	13.0	37.7	350	204	1.75490	325.920	7.76000	23.6174
36	.	.	.	.	.	0.75	40	12.6	26.0	.	.	.	232.800	5.82000	18.4762
37	16	100	75	45	60	0.70	37	12.4	33.0	230	203	1.17241	217.280	5.87243	17.5226
38	18	88	95	60	52	0.83	39	13.6	26.0	225	211	1.06635	257.632	6.60595	18.9435
39	.	.	.	.	.	0.60	34	9.5	4.0	.	.	.	186.240	5.47765	15.6062
40	18	82	98	60	36	0.50	33	9.7	6.0	174	204	0.85294	155.200	4.70303	16.0000
41	.	.	.	.	.	0.78	33	9.3	11.3	.	.	.	242.112	7.33673	26.0335
42	52	52	110	50	32	0.55	39	12.0	15.1	180	234	0.80342	170.720	4.37744	14.2267
43	.	.	.	.	.	0.00	35	10.5	21.6	.	.	.	240.320	7.09406	23.6455
44	32	96	54	38	28	0.65	37	11.9	27.4	206	208	0.99038	201.160	5.45297	16.9566
45	.	.	.	.	.	0.60	34	10.9	28.3	.	.	.	186.240	5.47765	17.0062
46	31	83	54	32	17	0.60	33	10.5	25.0	223	188	1.18617	186.240	5.64364	17.1321
47	.	.	.	.	.	0.50	35	11.5	30.6	.	.	.	155.200	4.43429	13.4557
48	12	98	104	52	34	0.55	35	11.3	27.3	250	218	1.14679	170.720	4.87771	15.1080
49	.	.	.	.	.	0.63	37	11.7	34.2	.	.	.	195.552	5.20519	16.7130
50	30	60	76	62	25	0.60	37	11.9	40.8	315	210	1.50000	211.872	5.72665	17.7211

EFFECT OF LEAD ON BONE MARROW AND PERIPHERAL BLOOD PROFILES

14:36 WEDNESDAY, AUGUST 5, 1970

OBS	DOG	DAY	GRP	TRT	RBC	WBC	MBLST	PMBLST	METAN	METAB	METAE	BANDN	BANDB	BANDE	SEGN	SEGB	SEGE	RBLST
51	30	84	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
52	30	91	2	1	5420	12600	10	28	64	0	0	72	0	0	104	0	2	8
53	30	98	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
54	30	105	2	0	5260	10300	20	34	56	0	4	96	0	6	140	0	C	12
55	30	112	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
56	30	119	2	0	5150	11000	10	21	40	0	2	82	0	0	98	0	0	10
57	30	126	2	0	5460	10300	13	19	33	0	0	85	0	3	90	0	2	15
58	31	0	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
59	31	7	5	0	4300	16800	14	16	16	0	0	62	0	0	102	0	0	14
60	31	14	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
61	31	21	5	1	4660	13900	20	24	28	0	2	84	4	8	102	6	2	26
62	31	28	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
63	31	35	5	1	4640	11500	16	28	50	0	2	60	0	2	94	0	2	36
64	31	42	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
65	31	49	5	1	4210	13400	18	30	50	0	4	58	0	3	104	0	0	32
66	31	56	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
67	31	63	5	1	4510	11100	20	25	20	0	0	45	0	5	145	0	5	16
68	31	70	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
69	31	77	5	1	4420	11300	30	36	36	C	0	58	0	0	96	0	0	14
70	31	84	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
71	31	91	5	1	4770	13000	18	23	51	C	4	75	0	4	95	0	4	10
72	31	98	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
73	31	105	5	0	4960	10400	16	19	32	0	0	90	0	2	86	0	2	18
74	31	112	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
75	31	119	5	0	5410	11400	14	24	30	0	0	84	0	4	92	0	2	10

OBS	PRORCYT	RCYTE	MRCYTE	MONO	UNDIF	ABS	PCV	GMHB	LEAD	MSERIS	ESERIS	ME	ABSI	GSHI	GSH2
51	.	.	.	.	.	0.76	37	11.5	54.1	.	.	.	235.504	6.37578	20.5134
52	6	82	116	90	42	0.93	39	12.0	61.5	200	212	1.32075	288.672	7.40185	22.5525
53	.	.	.	.	.	0.82	36	11.1	50.1	.	.	.	254.528	7.07022	22.9305
54	22	112	66	60	48	0.90	39	13.0	38.3	356	212	1.67925	279.360	7.16308	21.4852
55	.	.	.	.	.	0.70	40	12.2	35.0	.	.	.	211.200	5.43200	16.4606
56	14	90	92	55	30	0.90	36	12.8	20.0	253	206	1.22816	279.360	7.76000	21.8250
57	23	79,	88	60	20	0.73	39	12.8	28.0	245	205	1.19512	226.592	5.81005	17.7025
58	.	.	.	.	.	0.55	36	10.7	6.4	.	.	.	170.120	4.74222	15.9551
59	26	72	92	40	15	0.45	32	9.9	6.1	210	204	1.02941	139.600	4.36500	14.1051
60	.	.	.	.	.	0.70	33	9.7	19.6	.	.	.	217.280	6.58424	22.4000
61	22	108	58	62	30	0.40	34	10.3	22.9	200	214	1.30041	124.160	3.65176	12.0544
62	.	.	.	.	.	0.73	34	10.2	22.5	.	.	.	226.592	6.66441	22.2149
63	20	90	56	59	34	0.65	33	10.5	40.3	254	210	1.20952	201.760	6.11394	18.2152
64	.	.	.	.	.	0.63	34	10.3	57.7	.	.	.	195.552	5.75153	18.9856
65	35	88	60	30	29	0.75	33	10.2	62.8	267	215	1.24186	232.800	7.05455	22.8235
66	.	.	.	.	.	0.55	33	10.9	60.4	.	.	.	170.120	5.17333	15.6624
67	45	78	64	46	28	0.55	33	10.3	68.1	265	203	1.30542	170.720	5.17333	16.5740
68	.	.	.	.	.	0.65	37	10.9	61.2	.	.	.	201.760	5.45297	18.5101
69	17	60	95	48	36	0.60	33	10.2	72.0	296	186	1.59140	186.240	5.64364	18.2580
70	.	.	.	.	.	0.77	35	10.9	90.7	.	.	.	239.008	6.82880	21.9273
71	20	72	98	30	44	0.77	35	10.3	80.8	274	230	1.37000	239.008	6.82880	23.2047
72	.	.	.	.	.	0.67	34	10.5	60.5	.	.	.	207.560	6.11671	19.0065
73	24	80	91	55	38	1.00	38	11.7	59.6	248	213	1.16432	310.400	8.16842	26.5299
74	.	.	.	.	.	0.73	38	12.2	48.0	.	.	.	226.592	5.96295	18.5131
75	17	94	82	60	28	0.85	34	11.7	43.0	250	203	1.23153	263.840	7.76000	22.8504

EFFECT OF LEAD ON BONE MARROW AND PERIPHERAL BLOOD PROFILES

14:36 WEDNESDAY, AUGUST 9, 1978

OBS	DOG	DAY	GRP	TRT	RBC	WBC	RBLST	PMNLST	METAN	METAB	METAE	BANDN	BANDD	BANDE	SEGN	SEGB	SEGE	RBLST
76	31	126	5	0	5550	12200	12	18	26	0	0	08	0	2	86	0	4	12
77	37	0	5	0	4750	19900	6	12	22	0	0	92	0	0	98	0	0	4
78	37	7	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
79	37	14	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
80	37	21	5	1	4560	10600	26	28	22	C	0	66	2	0	90	2	0	12
81	37	28	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
82	37	35	5	1	4810	13400	10	12	42	C	0	48	0	0	126	0	0	18
83	37	42	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
84	37	49	5	1	4150	9800	20	15	50	0	0	54	0	0	130	0	0	21
85	37	56	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
86	37	63	5	0	4480	9200	10	40	50	0	1	82	0	8	100	.	0	10
87	37	70	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
88	37	77	5	0	4290	9000	22	30	36	C	0	80	0	2	114	0	0	12
89	37	84	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
90	37	91	5	0	4770	13000	0	24	62	C	0	72	0	2	86	0	0	8
91	37	98	5	C	5030	9300	10	26	66	C	0	75	0	4	80	0	3	10
92	37	105	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
93	37	112	5	0	4740	9000	11	20	58	C	0	8C	0	3	90	0	1	14
94	37	119	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
95	37	126	5	0	5440	9400	13	21	48	C	0	03	0	2	92	0	4	15
96	38	0	5	C	4070	26500	14	14	22	0	0	110	0	0	92	0	C	10
97	38	7	5	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
98	38	14	5	1	4220	11800	14	16	20	2	0	58	0	0	110	1	0	12
99	38	21	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
100	38	28	5	1	4240	13400	32	28	34	4	0	82	2	6	98	4	4	18

OBS	PRORCYT	RCYTE	NRCYTE	NCNO	BNDEF	ADS	PCV	GMHB	LEAD	MSERIS	ESERIS	DE	ADS1	GSH1	GSD2
76	20	80	96	50	32	0.65	30	12.4	41.C	236	216	1.09259	201.760	5.30947	15.0567
77	12	82	112	40	34	0.60	35	10.5	4.0	230	210	1.09524	186.240	5.32114	17.7371
78	.	.	.	.	.	0.60	33	9.9	6.4	.	.	.	186.240	5.64364	18.8121
79	.	.	.	.	.	0.80	33	10.2	27.4	.	.	.	248.320	7.52485	24.3451
80	20	106	70	50	30	0.50	33	10.0	21.6	256	208	1.23077	155.200	4.70303	15.5200
81	.	.	.	.	.	0.00	34	10.7	28.7	.	.	.	240.320	7.30353	23.2075
82	12	90	114	46	19	0.75	35	12.0	95.1	246	234	1.05128	232.800	6.65143	19.4000
83	.	.	.	.	.	0.73	41	12.4	107.3	.	.	.	226.592	5.52663	18.2135
84	11	08	98	60	42	0.70	35	14.C	115.6	277	218	1.27064	217.280	6.20800	15.5200
85	.	.	.	.	.	0.70	35	10.3	58.9	.	.	.	217.28C	6.20800	21.0951
86	16	00	104	55	40	C.60	33	10.3	51.6	299	210	1.42301	186.240	5.64364	18.0616
87	.	.	.	.	.	0.73	33	9.9	53.6	.	.	.	226.592	6.86642	22.8881
88	24	74	130	49	25	0.55	34	10.5	48.3	204	210	1.35238	294.800	8.67294	20.0838
89	.	.	.	.	.	0.80	33	10.3	53.9	.	.	.	248.320	7.52485	24.1007
90	14	100	108	62	32	0.75	33	10.7	44.3	254	230	1.10435	232.800	7.05455	21.7570
91	15	93	113	58	42	0.72	36	11.1	33.3	264	231	1.14286	223.488	6.20800	20.1351
92	.	.	.	.	.	1.CO	40	12.0	51.3	.	.	.	310.400	7.76000	24.2500
93	16	100	50	60	48	0.70	38	12.2	37.0	263	220	1.15351	217.280	5.71705	17.8058
94	.	.	.	.	.	0.75	34	12.0	35.C	.	.	.	232.800	6.84708	19.4000
95	19	97	02	58	45	0.80	38	12.4	28.0	263	213	1.23474	248.320	6.53474	20.0250
96	28	82	83	30	20	C.55	33	9.1	7.7	252	211	1.19431	170.720	5.17333	18.7604
97	.	.	.	.	.	C.50	31	9.1	6.4	.	.	.	155.200	5.00645	17.0549
98	32	70	54	37	15	0.70	32	9.5	42.2	221	176	1.25568	217.280	6.79000	22.8716
99	.	.	.	.	.	C.40	32	9.7	31.2	.	.	.	140.992	4.65600	15.3600
100	36	74	112	46	20	0.00	33	10.0	31.2	294	240	1.22500	248.320	7.52485	24.0320

OBS	DOG	DAY	GRP	INT	RBC	WBC	MOLST	PMOLST	METAM	METAB	METAE	BANDM	BANDB	BANDE	SEGM	SEGB	SEGE	BALST
101	38	35	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
102	38	42	5	1	4300	11400	10	14	28	0	2	76	0	0	126	0	0	6
103	38	49	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
104	38	56	5	1	4490	12300	10	18	22	0	0	48	0	0	121	0	0	5
105	38	63	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
106	38	70	5	1	4400	20800	20	28	50	0	0	100	0	6	110	0	0	10
107	38	77	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
108	38	84	5	1	4590	14900	10	22	36	0	0	116	0	6	136	0	2	4
109	38	91	5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
110	38	98	5	2	4390	10900	8	20	62	0	0	118	0	4	138	0	6	12
111	38	105	5	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
112	38	112	5	2	5130	9700	4	21	30	0	0	102	0	2	128	0	4	6
113	38	119	5	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
114	38	126	5	2	5130	11500	0	20	32	0	0	95	0	3	113	0	1	0
115	39	0	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
116	39	7	0	0	4120	15600	16	12	30	0	0	60	2	0	56	4	0	0
117	39	14	0	0	4190	10500	10	12	30	0	0	60	2	0	76	4	0	10
118	39	21	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
119	39	28	0	0	4200	12600	.	.	.	.	.	.	.	.	.	.	.	.
120	39	35	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
121	39	42	0	0	4150	9100	16	16	34	0	0	72	0	0	104	0	0	24
122	39	49	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
123	39	56	0	0	4130	9200	16	32	54	0	0	92	0	4	120	0	4	25
124	39	63	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
125	39	70	0	0	4550	10500	12	32	42	0	1	60	0	3	80	0	0	24

OBS	PRORCYT	RCYTE	MRCYTE	MGNO	UNDIF	AOS	PCV	GMHB	LEAD	MSERIS	ESERIS	ME	ADSI	GSHI	GSH2
101	.	.	.	.	.	0.60	33	10.5	54.4	.	.	.	211.072	6.32612	20.1021
102	12	88	122	50	21	0.65	33	10.3	84.1	256	220	1.12201	201.760	6.11394	19.5803
103	.	.	.	.	.	0.70	35	10.9	88.5	.	.	.	242.112	6.91749	22.2121
104	7	44	120	41	30	0.60	33	10.5	67.8	219	176	1.24432	186.240	5.64364	17.7371
105	.	.	.	.	.	0.70	36	11.3	102.5	.	.	.	217.280	6.03556	19.2283
106	20	72	116	55	10	0.70	34	10.3	86.8	314	218	1.44037	217.280	6.39059	21.0551
107	.	.	.	.	.	0.90	35	10.9	100.7	.	.	.	304.192	8.69120	27.9075
108	16	70	146	60	29	0.82	34	10.5	126.4	320	236	1.30903	254.528	7.48612	24.2408
109	.	.	.	.	.	0.82	32	10.3	110.9	.	.	.	254.528	7.95400	24.7115
110	22	78	90	19	17	0.73	33	9.9	113.8	356	202	1.76230	226.592	6.86642	22.0881
111	.	.	.	.	.	1.05	36	11.5	76.8	.	.	.	325.920	9.05333	28.3409
112	16	90	110	52	10	0.78	30	12.0	57.0	291	230	1.26522	242.112	8.27136	20.1760
113	.	.	.	.	.	0.85	32	11.1	46.0	.	.	.	263.040	8.24500	23.7694
114	10	89	98	58	49	0.78	37	12.4	42.0	272	213	1.27700	242.112	6.54357	19.5252
115	.	.	.	.	.	0.55	33	9.3	6.8	.	.	.	170.720	5.17333	10.3570
116	16	90	86	43	10	0.45	30	8.5	6.4	188	200	0.94000	139.680	4.65600	16.4329
117	14	94	88	50	20	0.75	30	8.8	5.5	194	206	0.94175	232.000	7.76000	26.4545
118	.	.	.	.	.	0.55	31	9.1	4.8	.	.	.	170.720	5.50710	18.7404
119	.	.	.	.	.	0.70	32	9.1	4.2	.	.	.	242.112	7.56600	26.6057
120	.	.	.	.	.	0.60	31	9.9	4.8	.	.	.	186.240	6.00774	18.8121
121	46	100	74	52	25	0.60	34	10.2	4.8	242	252	0.96032	186.240	5.47765	18.2588
122	.	.	.	.	.	0.63	32	9.9	4.8	.	.	.	195.552	6.11100	19.7527
123	50	81	115	43	32	0.50	31	11.1	4.2	322	277	1.16245	155.200	5.00645	13.2650
124	.	.	.	.	.	0.60	33	10.2	6.5	.	.	.	186.240	5.64364	18.2588
125	36	82	70	38	20	0.73	35	10.7	8.3	231	212	1.08962	226.592	6.47406	21.1160

EFFECT OF LEAD ON BONE MARROW AND PERIPHERAL BLOOD PROFILES

14:36 WEDNESDAY, AUGUST 9, 1978

OBS	DIG	DAY	GRP	TRT	RBC	WBC	MBLST	PMBLST	METAN	METAB	METAE	BANDN	BANDB	BANDE	SEGN	SEGB	SEGE	RBLST
126	39	77	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
127	39	84	0	0	5020	11000	12	26	46	0	0	74	0	4	76	0	5	10
128	39	91	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
129	39	98	0	2	5000	10900	6	14	38	0	0	74	0	0	106	0	2	4
130	39	105	0	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
131	39	112	0	2	5310	11800	4	10	62	0	2	68	0	6	74	0	10	4
132	39	119	0	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
133	39	126	0	2	5140	9300	8	10	33	0	1	71	0	3	80	0	2	10
134	40	0	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
135	40	7	2	0	4070	14200	4	14	12	4	0	74	0	0	90	4	0	6
136	40	14	2	1	4000	11900	10	18	16	0	0	70	0	0	70	2	0	10
137	40	21	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
138	40	28	2	1	4780	9900	6	10	18	2	2	54	0	0	106	0	4	2
139	40	35	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
140	40	42	2	1	4910	9900	18	30	30	0	0	70	0	2	126	0	6	10
141	40	49	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
142	40	56	2	1	4670	9600	14	28	26	0	0	80	2	0	90	0	0	20
143	40	63	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
144	40	70	2	1	5180	7700	26	24	44	0	2	56	0	0	104	0	10	22
145	40	77	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
146	40	84	2	1	4840	8100	12	32	52	0	0	110	0	6	108	0	0	20
147	40	91	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
148	40	98	2	0	4960	7900	6	16	34	0	0	40	0	4	114	0	6	6
149	40	105	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
150	40	112	2	0	5090	8200	0	10	32	0	0	48	0	2	112	0	4	6

OBS	PRORCYT	RCYTE	NRCYTE	MCNO	UNDIF	AOS	PCV	GMHB	LEAD	MSERIS	ESERIS	ME	AOSI	GSHI	CSH2
126	.	.	.	.	.	0.64	33	11.7	6.4	.	.	.	190.656	6.01500	18.9751
127	16	120	62	58	23	0.79	38	11.7	7.8	243	208	1.16827	245.216	6.45305	20.9586
128	.	.	.	.	.	0.86	36	11.5	11.2	.	.	.	266.544	7.41511	23.2125
129	18	106	88	52	26	0.78	36	11.9	10.8	240	216	1.11111	242.112	6.72533	20.3455
130	.	.	.	.	.	1.00	40	10.9	3.5	.	.	.	310.400	7.76000	28.4771
131	12	112	84	42	20	0.80	30	12.2	6.0	236	212	1.11321	248.320	6.53474	20.3541
132	.	.	.	.	.	0.65	35	11.9	4.0	.	.	.	201.760	5.76457	16.9546
133	20	96	85	60	37	0.80	37	12.0	5.0	216	211	1.02370	248.320	6.71135	20.6533
134	.	.	.	.	.	0.55	34	9.9	6.8	.	.	.	170.720	5.02118	17.2444
135	12	86	98	44	26	0.45	31	9.1	7.1	202	202	1.00000	139.680	4.50581	15.3495
136	10	90	74	62	40	0.75	32	9.5	16.1	194	200	0.97000	232.800	7.27500	24.5053
137	.	.	.	.	.	0.50	32	9.9	22.5	.	.	.	155.200	4.85000	15.6168
138	10	86	102	50	37	0.80	34	10.5	26.1	202	208	0.97115	248.320	7.30353	23.6495
139	.	.	.	.	.	0.60	32	10.5	34.8	.	.	.	186.240	5.82000	17.7211
140	38	92	94	44	30	0.60	36	11.3	30.0	290	234	1.23932	186.240	5.17333	16.4014
141	.	.	.	.	.	0.70	35	11.1	31.6	.	.	.	217.280	6.20000	19.5748
142	46	80	64	50	19	0.60	35	11.7	34.6	248	210	1.18095	186.240	5.32114	15.9119
143	.	.	.	.	.	0.50	35	11.1	37.7	.	.	.	100.032	5.14377	16.2191
144	28	68	104	30	29	0.70	38	11.9	34.2	314	222	1.41441	217.280	5.71785	18.2588
145	.	.	.	.	.	0.70	36	12.0	37.1	.	.	.	217.280	6.03556	18.1067
146	22	110	62	50	28	1.00	37	11.7	57.3	320	222	1.44144	310.400	8.38919	26.5259
147	.	.	.	.	.	0.81	36	11.5	46.2	.	.	.	270.048	7.50133	23.4824
148	26	84	85	38	28	0.93	37	12.0	44.3	220	201	1.09453	288.672	7.80195	24.0560
149	.	.	.	.	.	1.03	40	13.6	32.5	.	.	.	319.112	7.97800	23.5682
150	20	83	90	40	30	0.83	41	13.6	27.0	224	199	1.12563	257.632	6.28371	18.9435

EFFECT OF LEAD ON BONE MARROW AND PERIPHERAL BLOOD PROFILES

14:36 WEDNESDAY, AUGUST 9, 1978

OBS	DOG	DAY	GRP	TMT	RBC	WBC	NBLST	PMBLST	MCIAN	METAB	METAE	BANDN	BANDB	BANDE	SEGN	SEGB	SEGE	ABLST
151	40	119	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
152	40	126	2	0	5960	7400	10	26	40	0	0	70	0	0	80	0	2	8
153	42	0	0	0	4590	19700	4	14	22	0	6	100	0	2	90	2	0	4
154	42	7	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
155	42	14	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
156	42	21	0	0	4760	13200	10	14	16	4	0	84	0	0	102	2	2	24
157	42	28	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
158	42	35	0	0	4400	12400	10	16	30	0	2	76	0	0	80	0	0	20
159	42	42	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
160	42	49	0	0	4740	12000	11	19	28	1	0	72	0	0	81	0	0	19
161	42	56	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
162	42	63	0	0	5020	13000	18	40	36	0	0	00	0	4	94	0	6	26
163	42	70	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
164	42	77	0	0	5610	12600	12	22	41	0	0	72	0	0	90	0	0	30
165	42	84	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
166	42	91	0	0	5040	13600	17	20	32	0	0	82	0	0	86	0	3	25
167	42	98	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
168	42	105	0	0	4840	9900	20	14	30	0	2	03	0	3	90	0	0	10
169	42	112	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
170	42	119	0	0	5090	11100	18	16	32	0	2	79	0	4	96	0	0	20
171	42	126	0	0	5710	11400	10	15	20	0	0	60	0	0	90	0	4	16
172	43	0	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
173	43	7	2	0	3900	13900	10	14	20	2	0	70	6	0	80	0	0	8
174	43	14	2	1	4000	11900	0	26	26	0	0	114	0	0	112	4	0	14
175	43	21	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.

OBS	PRORCYT	RCYTE	MRCYTE	MCRO	UNDIF	ABS	PCV	GUNB	LEAD	MSERIS	ESERIS	ME	ABSI	GSHI	GSH2
151	.	.	.	.	.	0.75	38	13.2	32.0	.	.	.	232.000	6.12632	17.6264
152	10	00	92	50	42	0.00	39	12.8	26.0	220	206	1.10600	240.320	6.36718	15.4000
153	10	92	94	49	15	0.50	35	10.0	6.8	240	200	1.20000	155.200	4.43425	15.5200
154	.	.	.	.	.	0.40	31	9.7	6.4	.	.	.	124.160	4.00516	12.8000
155	.	.	.	.	.	0.70	33	10.0	5.1	.	.	.	217.280	6.58424	21.7280
156	40	102	46	42	10	0.52	35	11.3	4.2	234	212	1.10377	161.408	4.61166	14.2839
157	.	.	.	.	.	0.75	34	10.2	4.8	.	.	.	232.800	6.84706	22.0215
158	24	90	70	50	35	0.60	35	10.9	7.1	222	204	1.00024	186.240	5.32114	17.0662
159	.	.	.	.	.	0.63	37	11.9	6.1	.	.	.	195.552	5.28515	16.4329
160	27	93	75	61	34	0.59	35	11.1	5.5	218	216	1.00926	170.720	4.87771	15.3802
161	.	.	.	.	.	0.65	36	12.4	7.4	.	.	.	201.760	5.60444	16.2710
162	48	04	04	48	30	0.70	36	11.9	10.9	270	242	1.14876	217.280	6.03556	18.2500
163	.	.	.	.	.	0.75	39	12.6	6.8	.	.	.	232.800	5.96923	18.4462
164	30	50	76	50	43	0.70	39	12.8	11.2	237	202	1.17327	217.280	5.57128	16.9150
165	.	.	.	.	.	0.92	39	12.4	13.9	.	.	.	285.568	7.32226	23.0297
166	32	00	90	65	50	0.95	41	14.0	10.8	240	227	1.05727	294.800	7.19220	21.0629
167	.	.	.	.	.	0.93	39	12.2	5.3	.	.	.	288.672	7.40185	23.6616
168	37	08	100	59	50	0.80	39	12.8	6.1	242	243	0.99508	273.152	7.00390	21.3600
169	.	.	.	.	.	0.80	40	13.2	7.0	.	.	.	248.320	6.20800	18.8121
170	35	90	105	65	45	0.70	38	12.8	6.0	247	250	0.98800	217.280	5.71789	16.9750
171	30	92	82	50	35	0.80	39	13.6	12.0	227	220	1.03182	248.320	6.36718	18.2588
172	.	.	.	.	.	0.45	32	8.7	6.8	.	.	.	139.680	4.36500	16.0552
173	24	04	04	60	30	0.40	31	8.9	7.4	210	200	1.05000	124.160	4.00516	13.5506
174	24	124	58	54	15	0.75	31	9.1	23.0	290	220	1.31818	232.800	7.50968	25.5824
175	.	.	.	.	.	0.50	34	10.3	26.1	.	.	.	180.032	5.29506	17.4780

EFFECT OF LEAD ON BONE MARROW AND PERIPHERAL BLOOD PROFILES

14:36 WEDNESDAY, AUGUST 5, 1978

OBS	DOG	DAY	GRP	TMT	RBC	WBC	MBLST	PMBLST	METAN	METAB	METAE	BANDN	BANDB	BANDE	SEGN	SEGB	SEGE	RBLST
176	43	28	2	1	4640	15600	12	12	30	4	4	90	0	4	102	0	6	18
177	43	35	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
178	43	42	2	1	4640	16400	8	10	20	0	0	86	0	0	126	0	0	20
179	43	49	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
180	43	56	2	1	4840	15500	10	12	28	0	0	76	0	2	106	0	0	24
181	43	63	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
182	43	70	2	1	4920	13800	14	28	36	0	0	80	0	4	115	0	2	20
183	43	77	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
184	43	84	2	1	5390	15600	18	28	52	0	0	122	0	0	148	0	0	30
185	43	91	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
186	43	98	2	2	5500	14900	12	22	64	0	0	92	0	4	106	0	0	8
187	43	105	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
188	43	112	2	2	5270	15500	8	28	40	0	0	74	0	4	140	0	6	10
189	43	119	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
190	43	126	2	2	5000	13000	10	18	30	0	0	86	0	2	82	0	2	6

OBS	PRORCYT	RCYTE	MRCYTE	MONO	UNDIF	ABS	PCV	GMHB	LEAD	MSERIS	ESERIS	ME	ABSI	GSHI	GSH2
176	42	76	80	46	28	0.70	33	10.5	24.8	264	216	1.22222	217.280	6.58424	20.6533
177	.	.	.	.	.	0.50	33	10.5	26.7	.	.	.	155.200	4.70303	14.7810
178	26	96	72	40	30	0.60	34	10.5	28.3	250	214	1.16822	186.240	5.47765	17.0862
179	.	.	.	.	.	0.63	35	11.1	44.5	.	.	.	195.552	5.58720	17.6173
180	28	52	100	57	25	0.55	36	12.4	41.3	234	204	1.14706	170.720	4.74222	13.7677
181	.	.	.	.	.	0.50	36	11.7	62.6	.	.	.	155.200	4.31111	13.2650
182	44	89	66	52	30	0.55	37	11.7	46.3	279	219	1.27397	170.720	4.61405	14.5515
183	.	.	.	.	.	0.65	35	12.2	56.1	.	.	.	201.760	5.76457	16.5377
184	38	116	54	48	20	0.82	38	12.4	72.4	368	238	1.54622	254.528	6.69811	20.5265
185	.	.	.	.	.	0.75	38	12.2	48.1	.	.	.	232.800	6.12632	19.0820
186	10	96	104	20	18	0.64	40	13.0	53.6	300	218	1.37615	198.656	4.96640	15.2812
187	.	.	.	.	.	0.80	38	12.2	33.0	.	.	.	248.320	6.53474	20.3541
188	14	118	98	44	28	0.60	39	12.4	32.0	300	240	1.25000	186.240	4.77538	15.0194
189	.	.	.	.	.	0.85	37	13.2	35.0	.	.	.	263.840	7.13081	19.9079
190	16	83	90	50	23	0.70	40	14.0	40.0	230	195	1.17949	217.280	5.43200	15.5200

DOG No. 39
(Group 1 = Control)

Day	BWT	No. of marrow cells with iron granules
0	5.0	o
14	7.0	+
28	9.0	o
42	11.0	o
56	13.0	o
70	16.0	o
84	17.5	o
98	19.0	+
112	21.0	o
126	--	+

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

DOG No. 42
(Group 1 = Control)

Day	BWT	No. of marrow cells with iron granules
0	10.0	o
14	14.0	+
28	17.5	o
42	20.5	o
56	23.0	+
70	26.0	+
84	28.0	o
98	30.0	o
112	31.0	o
126	--	+

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

DOG No. 29
(Group 2)*

Day	BWT	No. of marrow cells with iron granules
0	9.0	o
14	11.5	o
28	14.0	+
42	16.0	+
56	18.0	o
70	20.0	++
84	21.0	+++
98	22.0	+
112	24.0	++
126	--	++

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

*Group 2 = 2 mg Pb/kg.

DOG No. 43
(Group 2)*

Day	BWT	No. of marrow cells with iron granules
0	7.0	o
14	9.0	o
28	10.0	+
42	13.0	+
56	14.0	o
70	16.0	++
84	17.0	++
98	18.0	++
112	19.0	+
126	--	++

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

*Group 2 = 2 mg Pb/kg.

DOG No. 30
(Group 2)*

Day	BWT	No. of marrow cells with iron granules
0	7.0	o
14	9.0	+
28	11.0	o
42	13.0	o
56	14.5	o
70	16.0	o
84	17.0	++
98	18.0	+++
112	19.0	+
126	--	o

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

*Group 2 = 2 mg Pb/kg.

DOG No. 40
(Group 2)*

Day	BWT	No. of marrow cells with iron granules
0	7.0	o
14	8.0	o
28	10.5	o
42	13.0	o
56	14.0	+
70	18.0	+
84	19.0	o
98	21.0	++
112	21.0	++
126	--	+

Day = Day of experiment

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

*Group 2 = 2 mg Pb/kg.

DOG No. 31
(Group 3)*

Day	BWT	No. of marrow cells with iron granules
0	10.0	o
14	11.0	o
28	13.0	o
42	15.0	o
56	15.0	o
70	17.0	++
84	19.0	+
98	19.0	++++
112	21.0	++
126	--	++

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

*Group 3 = 5 mg Pb/kg.

DOG No. 37
(Group 3)*

Day	BWT	No. of marrow cells with iron granules
0	7.0	o
14	10.5	o
28	12.5	o
42	12.5	o
56	9.5	++
70	14.5	o
84	18.0	+
98	21.0	o
112	23.0	+
126	--	+

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

*Group 3 = 5 mg Pb/kg.

DOG No. 28
(Group 3)*

Day	BWT	No. of marrow cells with iron granules
0	9.5	o
14	12.5	o
28	15.0	o
42	16.5	+
56	17.5	o
70	19.5	+++
84	20.5	++
98	21.0	++++
112	22.0	+++
126	--	++

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

*Group 3 = 5 mg Pb/kg.

DOG No. 38
(Group 3)*

Day	BWT	No. of marrow cells with iron granules
0	7.0	o
14	10.0	o
28	12.5	o
42	14.0	+
56	14.5	++
70	17.0	+
84	19.0	o
98	20.0	+++
112	23.0	++
126	--	++

Day = Day of experiment.

BWT = Body weight in lbs.

No. of marrow cells with iron granules = Number of cells with iron granules on staining with ferrocyanide and hydrochloric acid.

*Group 3 = 5 mg Pb/kg.

EXAMPLES OF BONE MARROW CELLS FROM EXPERIMENTAL ANIMALS

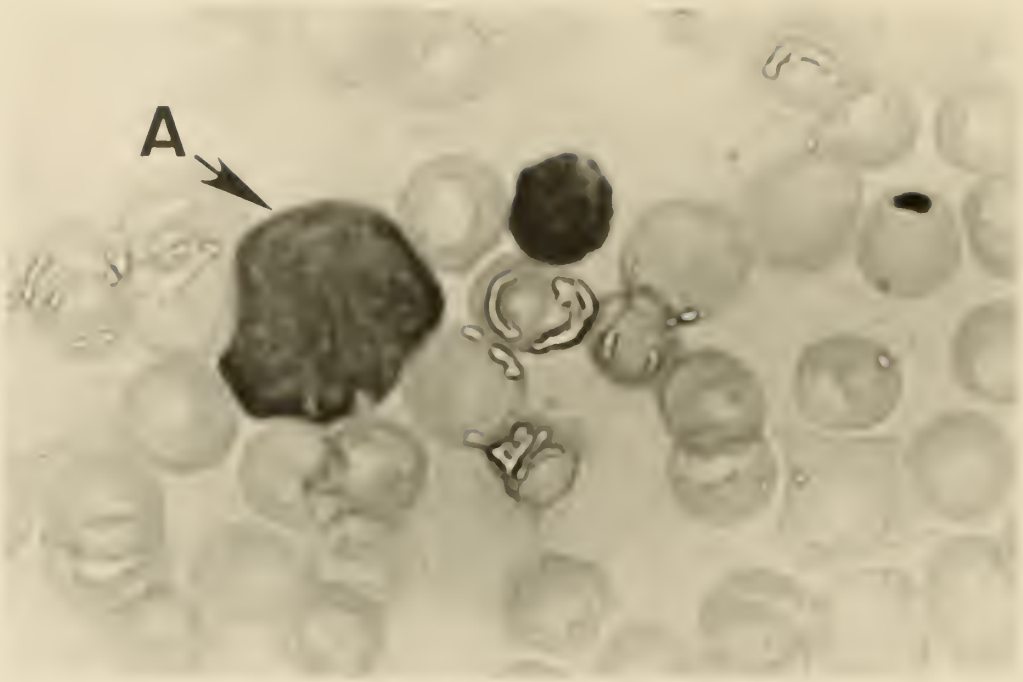

FIG. 2. Bone marrow smear on day 70 from one dog receiving 5 mg lead/kg daily. The cell lettered A is a myeloblast and an unlettered cell is a lymphocyte.

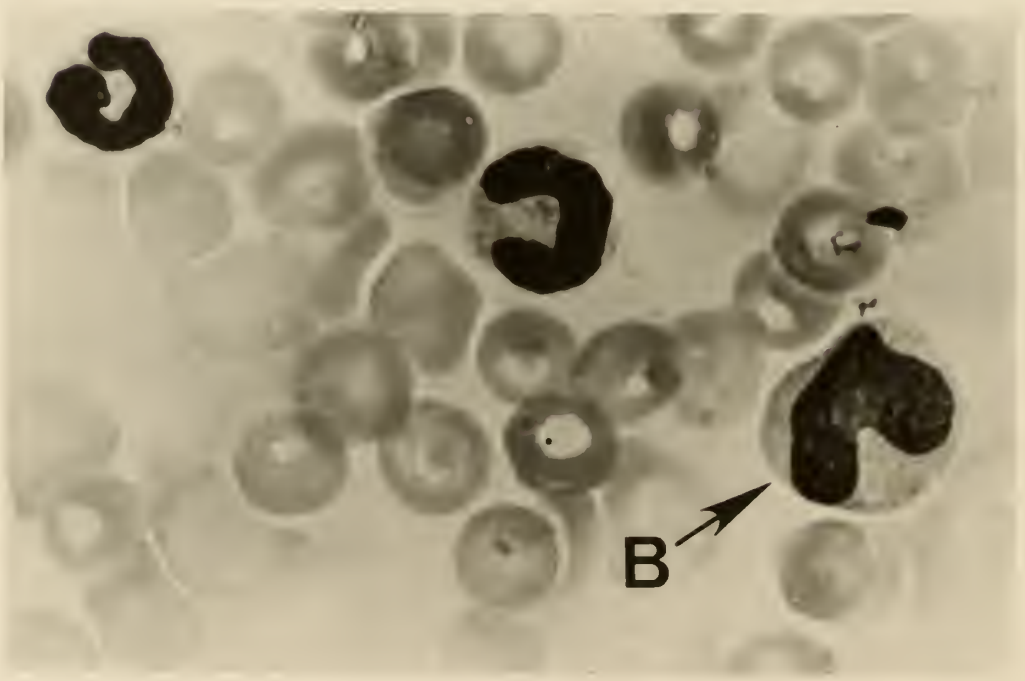

FIG. 3. Bone marrow smear on day 70 from one dog receiving 2 mg lead/kg/daily. The cell lettered B is a band neutrophil.

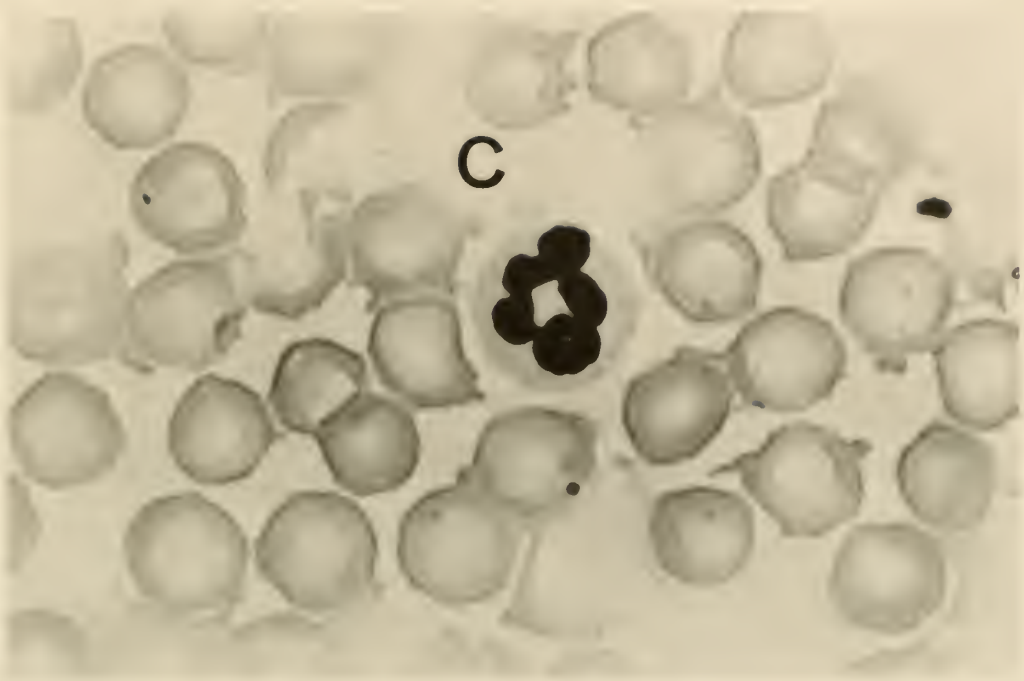

FIG. 4. Bone marrow smear on day 112 from one control dog. The cell lettered C is a hypersegmented neutrophil.

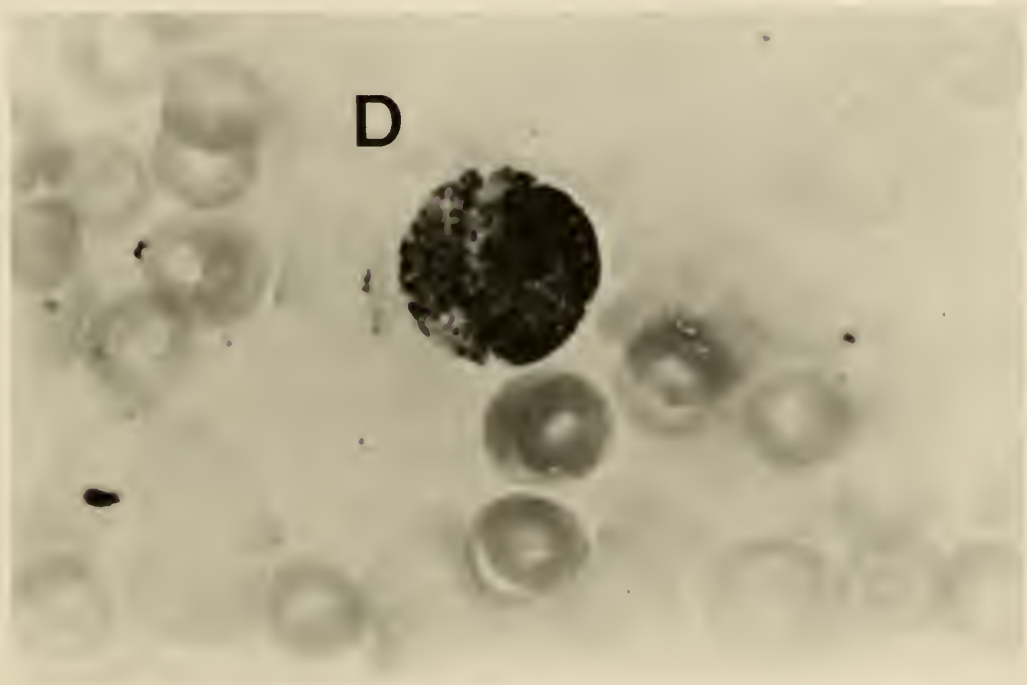

FIG. 5. Bone marrow smear on day 42 from one dog receiving 5 mg lead/kg/daily. The cell lettered D is an eosinophil.

FIG. 6. Marrow smear on day 98 from one dog receiving 2 mg lead/kg/daily. The cell lettered E is a rubriblast and the cell at the corner is a lymphocyte.

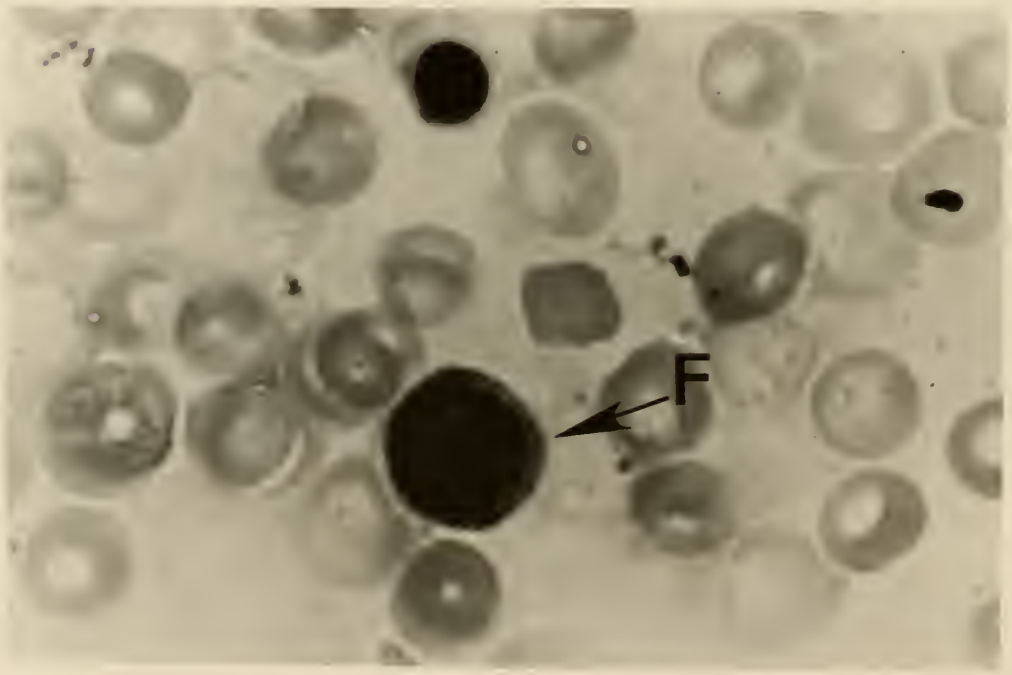

FIG. 7. Marrow smear on day 28 from one dog receiving 5 mg lead/kg/daily. The cell lettered F is a rubricyte and the unlettered cell at the top is a metarubricyte.

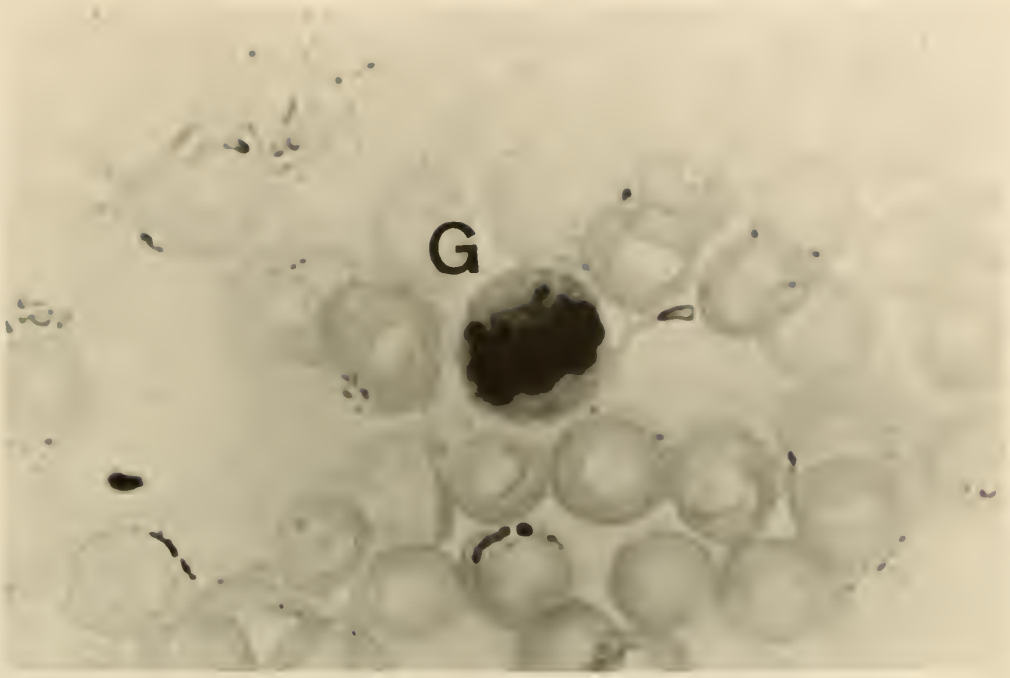

FIG. 8. Marrow smear on day 14 from one control dog. The cell lettered G is rubricyte in mitosis.

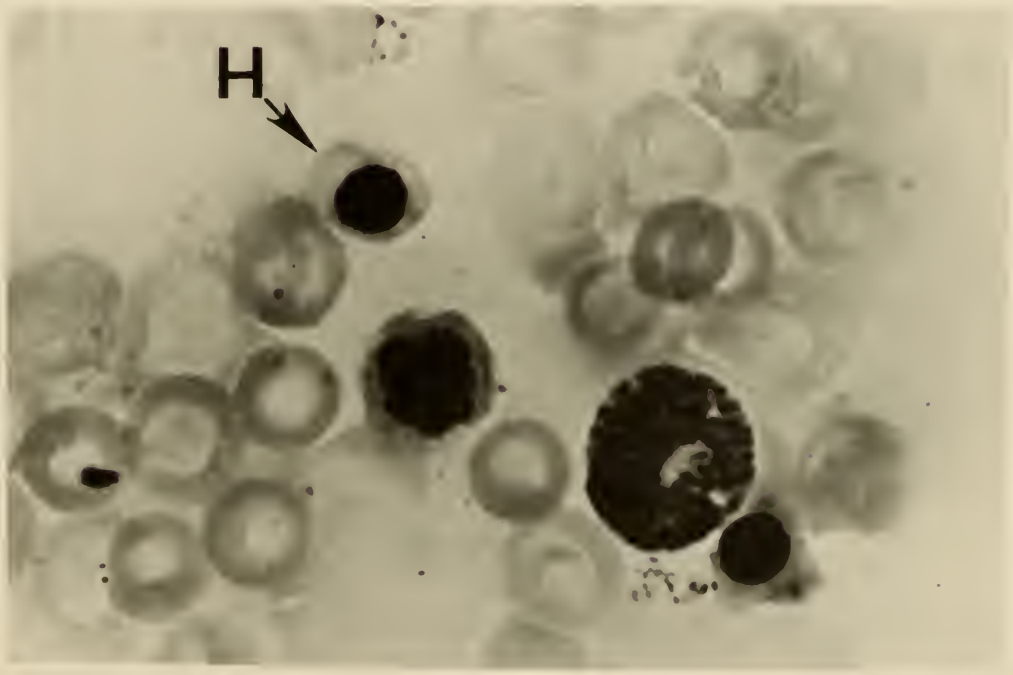

FIG. 9. Marrow smear on day 91 from one dog receiving 5 mg lead/kg. The lettered cell is a metarubricyte. The other unlettered cells include rubricyte, an eosinophil and a metarubricyte.

EFFECT OF CHRONIC LEAD EXPOSURE ON THE HEMATOLOGY, BLOOD GLUTATHIONE AND BONE MARROW OF DOGS

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

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1978

The undesirable environmental effects of lead have been recognized for many years in people and animals. A prominent effect of repeated lead exposure is the effect on erythropoiesis, expressed as anemia, basophilic stripping of red cells and increased number of nucleated red blood cells in the peripheral circulation. The purpose of this study was to evaluate the effect of chronic lead exposure on the hemopoietic system of dogs and to study the correlation between blood levels and hematologic, biochemical and bone marrow cytologic parameters.

The effects of oral lead exposure on canine hematology, blood glutathione and bone marrow cytology were studied in 10 dogs. Two dogs served as control, four received 2 mg lead (as acetate)/kg daily and four received 5 mg lead/kg daily for 13 weeks. At that time, one-half of each group was treated with calcium ethylene-diamine tetraacetate (EDTA). All animals were then monitored for another four weeks. Blood lead levels, hematology, and blood glutathione were measured weekly during the 18 weeks of study. Bone marrow cytology and iron granules in bone marrow cells were monitored on alternate weeks.

Clinical signs of toxicosis were observed after six weeks in only one dog in the high dose group. The signs included emaciation, anorexia, muscular weakness, loss of weight, evidence of abdominal pain and depression. There was no significant change in the blood glutathione activity of any group. There was an increase in nucleated red blood cells in the high lead dosed group and a trend toward depression of the packed

cell volume in the same group. Dogs receiving doses of lead had increases in bone marrow segmented neutrophils, had increased myeloid:erythroid (M:E) ratios, and had increased numbers of bone marrow cells with iron granules. Statistically, myeloid series cells and segmented neutrophils remained elevated in EDTA-treated animals than in those not treated with EDTA; however, this may not be of diagnostic significance. Blood lead levels, nucleated red blood cells and M:E ratios decreased after cessation of lead administration, and the number of marrow cells with iron granules showed a decreasing trend.

This study showed that the dosages of lead used altered bone marrow cytology, but had no effect on canine hematology and blood glutathione except for increasing the number of circulating nucleated red blood cells and a trend toward depression of packed cell volume.